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出版情報: Kyushu University, 2018, 博士(医学), 課程博士
バージョン:
権利関係:

https://hdl.handle.net/2324/2236084
Research paper

Tryptophan-kynurenine and lipid related metabolites as blood biomarkers for first-episode drug-naïve patients with major depressive disorder: An exploratory pilot case-control study

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Keywords:
First-episode drug-naïve major depressive disorder
Blood biomarkers
Tryptophan-kynurenine metabolites
High density lipoprotein-cholesterol
Cholesteryl esters

ABSTRACT

Background: Early intervention in depression has been critical to prevent its negative impact including suicide. Recent blood biomarker studies for major depressive disorder (MDD) have suggested that tryptophan-kynurenine and lipid related metabolites are involved in the pathophysiology of MDD. However, there have been limited studies investigating these blood biomarkers in first-episode drug-naïve MDD, which are particularly important for early intervention in depression.

Methods: As an exploratory pilot case-control study, we examined the above blood biomarkers, and analyzed how these biomarkers are associated with clinical variables in first-episode drug-naïve MDD patients, based on metabolome/lipidome analysis.

Results: Plasma tryptophan and kynurenine levels were significantly lower in MDD group (N = 15) compared to healthy controls (HC) group (N = 19), and plasma tryptophan was the significant biomarker to identify MDD group (area under the curve = 0.740). Lower serum high density lipoprotein-cholesterol (HDL-C) was the predictive biomarker for severity of depression in MDD group (R² = 0.444). Interestingly, depressive symptoms were variously correlated with plasma tryptophan-kynurenine and lipid related metabolites. Moreover, plasma tryptophan-kynurenine metabolites and cholesteryl esters (CEs) were significantly correlated in MDD group, but not in HC group.

Limitations: This study had small sample size, and we did not use the multiple test correction.

Conclusions: This is the first study to suggest that not only tryptophan-kynurenine metabolites but also HDL-C and CEs are important blood biomarkers for first-episode drug-naïve MDD patients. The present study sheds new light on early intervention in clinical practice in depression, and further clinical studies especially large-scale prospective studies are warranted.

ARTICLE INFO

1. Introduction

Major depressive disorder (MDD) is a common psychiatric disorder, and its prevalence is considered to be 8–12% worldwide (Andrade et al., 2003; Mitchell et al., 2009; Smith, 2014). Patients with MDD show high suicidal risk, severe impairment of social functioning, and cognitive dysfunction (Dong et al., 2017; Kuperberg et al., 2016; Mann et al., 2005; Rosenblat et al., 2015). Early intervention in depression is
critical for effective clinical practice, as longer duration of untreated depression is associated with poorer clinical outcomes including depression-related disability (Ghio et al., 2015). Therefore, early detection of persons with depression is warranted. The Structured Clinical Interview for DSM-IV-TR (SCID) is a well-established structured diagnostic instrument (First et al., 2002). Beck Depression Inventory-Second Edition (BDI-II) is utilized as one of the most reliable self-rated scales to assess severity of depression (SOD) (Beck et al., 1996). Present MDD diagnostic and SOD evaluation systems including SCID and BDI-II completely rely on persons’ subjective descriptions, spoken information, and attitudes. As a result, misdiagnoses based on such subjective information are unavoidable (Mitchell et al., 2009). Such difficulties have resulted in confusion in clinical practice (Kato et al., 2016; Kato and Kanba, 2017; Kato et al., 2011b; Kato et al., 2011c). Thus, to resolve the above limitations, the establishment of objective methods to detect persons with depression has been warranted. Biomarkers for MDD have been investigated by various research methods including brain imaging (Gadad et al., 2017; Gururajan et al., 2016; Kunugi et al., 2015). However, to our knowledge, blood biomarkers in practical use for depression have been very limited until now. In order to conduct early intervention in depression more effectively, discovering blood biomarkers for first-episode drug-naïve MDD patients is especially important; yet, many previous studies have included patients under medication and patients with recurrent episodes of MDD.

The tryptophan-kynurenine pathway has recently been highlighted in pathophysiological understanding of MDD beyond the traditional “serotonin hypothesis”, based on evidence such as serotonin deficiency by tryptophan reduction, and neurotoxicity due to altered kynurenine metabolites (Dantzer, 2017; Dantzer et al., 2008; Dantzer et al., 2011; Halaris, 2013, 2017; Maes et al., 2012; Maes et al., 2011b; Maes et al., 2009; Myint, 2012; Myint et al., 2007; Myint et al., 2012; Ormstad et al., 2016; Roomruangwong et al., 2017; Schwartz et al., 2012; Schwarz and Stone, 2017). On the other hand, a recent study has shown the significance of routine blood biochemical markers including high density lipoprotein-cholesterol (HDL-C) to identify patients with MDD (Peng et al., 2016). Thus, lipid related metabolites have also been suggested to be involved in the pathophysiology of MDD (Liu et al., 2016; Parekh et al., 2017; Shelton and Miller, 2010). Lipid species serve multiple important functions such as membrane composition, energy storage, and signal transduction (Fonteh et al., 2006; Liu et al., 2016; Liu et al., 2015b). However, despite biological significance of lipid species, much remains unknown about lipid species pathophysiology from tryptophan metabolites in MDD. A recent study, using tryptophan hydroxylase-2 knockout mouse, has shown that serotonin deficiency is caused by elevated oxidative stress associated with altered metabolism of serum lipid species (Weng et al., 2016). Thus, we have hypothesized that peripheral lipid species may link with brain systems through tryptophan metabolites in the pathophysiology of MDD.

To our knowledge, blood biomarker studies combining tryptophan metabolites, routine biochemical markers, and lipid species especially focusing on first-episode drug-naïve MDD patients have not existed until now. As an exploratory pilot case-control study between healthy controls (HC) and first-episode drug-naïve MDD patients, we herein investigated blood biomarkers associated with diagnosis, SOD, and depressive symptoms, using a combination of the above blood biomarkers, with metabolome/lipidome analysis. Moreover, we estimated the relationship between tryptophan metabolites and lipid species in MDD.

2. Methods

The present study was approved by the ethics committee of Kyushu University and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.1. Subjects

Healthy subjects and first-episode drug-naïve MDD patients were enrolled in the present study from May 2014 until December 2015. 19 healthy subjects were selected as HC by interview based on SCID regarding any previous or ongoing psychiatric disorder, physical disorder, and medications, which were set as exclusion criteria. Psychiatric patients were enrolled in Kyushu University Hospital and its related affiliations (mainly outpatients’ clinics). The diagnosis of MDD was determined by trained psychiatrists, according to SCID (First et al., 2002). We selected 15 first-episode drug-naïve MDD patients (9 males and 6 females). We confirmed that all the patients have no history of the following diseases; neurodegenerative disorders, bipolar or psychotic disorders, mental retardation, substance abuse, and physical disorders such as cardiovascular disease, liver and kidney disorder, infectious disease, malignancy, and head trauma. All peripheral venous blood samples were collected between 10:00 and 15:00. We did not check as to whether blood samples were collected under fasting or non-fasting conditions, since blood collection of first-episode drug-naïve MDD patients was performed according to the voluntary time when the patients consulted. The plasma and serum were immediately extracted and then frozen and stored at – 80 °C until required for analysis. On the day of peripheral venous blood collection, we assessed BDI-II to evaluate SOD and depressive symptoms (Beck et al., 1996). BDI-II is a self-rated scale of depression with relatively many questions on affective and cognitive aspects, which are core features of MDD and are related to negative issues, such as suicide (Beck et al., 1996; Green et al., 2015). In addition, a self-rated scale is useful to conduct early intervention not only in psychiatric settings but also primary care and corporate health settings. Therefore, we selected BDI-II as a rating scale of depression in the present study. The following severity range for BDI-II total score was used to classify SOD: mild depression (14–19); moderate depression (20–28); and severe depression (29–63) (Beck et al., 1996). BDI-II items were assigned to the following sub-categories: “affective” (items “sadness”, “loss of pleasure”, “crying”, and “indecisiveness”), “motional” (items “pessimism” and “suicidal thoughts”), “cognitive” (items “loss of interest” and “concentration difficulty”), “cognitive distortions” (items “past failure”, “guilty feelings”, “punishment feelings”, “self-dislike”, “self-criticalness”, and “worthlessness”), “behavioral” (items “agitation”, “loss of energy”, “irritability”, and “tiredness or fatigue”), and “vegetative” (items “changes in sleep”, “changes in appetite”, and “loss of interest in sex”) according to a previous study (Cohen, 2008).

2.2. Routine blood biochemical markers

Routine blood biochemical markers including serum total-cholesterol (Total-C), HDL-C, low density lipoprotein-cholesterol (LDL-C), fibrinogen (Fib), total-bilirubin (T-bil), direct-bilirubin (D-bil), indirect-bilirubin (I-bil), uric acid (UA), and high-sensitivity C-reactive protein (hsCRP) were measured by automatic biochemical analyzer (SRL, Inc., Tokyo, Japan).

2.3. Blood tryptophan metabolites (Metabolomics)

Plasma tryptophan and its related metabolites including tryptophan, serotonin, indoleacetaetate, indolecarboxaldehyde, kynurenine, kynurenic acid, and xanthurenic acid were measured by liquid chromatography-mass spectrometry (LC-MS) using LCMS-8040 (Shimadzu Corp., Kyoto, Japan), as described previously (Setoyama et al., 2016). Concentration of each plasma metabolite was determined using their standard reagents. Moreover, plasma kynurenine/tryptophan ratio was calculated as an indicator of tryptophan degradation, i.e. an indicator for the activity of tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) (Schroksmadel et al., 2006).
2.4. Blood lipid species (Lipidomics)

One-microliter of plasma was mixed with 1 mL of methanol. After centrifugation at maximum speed, two-microliter of the supernatant was subjected to LC-MS analysis employing a triple quadrupole mass spectrometer LCMS-8060 (Shimadzu Corp., Kyoto, Japan). High abundant 62 plasma lipid species including lysophosphatidylcholine (LPC), lyso phosphatidylethanolamine (LPE), free cholesterol, phosphatidylcholine (PC), phosphatidylethanolamine (PE), cholesteryl ester (CE), and triacylglycerol (TG) were separated by a reverse phase liquid chromatography using a KINETEX 2.6 µ C8 100 A column (15 × 2.1 mm, phenomenex). The mobile phase A consisted of 20 mM ammonium formate and the mobile phase B consisted of acetonitril: isopropanol (1:1). Column oven temperature was 40 °C. The gradient elution program was as follows: a flow rate of 0.3 mL/min: 0–1 min, 20%B; 1–2 min, 20–40%B (linear gradient); 2–25 min, 40–92.5%B (non-linear, “shark’s fin curve”); 25–26 min, 92.5–100%B (linear gradient); 26–35 min, 100%B; 35–35.1 min, 100–20%B; 35.1–38 min, 20%B. Parameters for positive electrospray ionization mode under multiple reaction monitoring were as follows; drying gas flow rate, 10 L/min; nebulizer gas flow rate, 3 L/min; heating gas flow rate, 10 L/min; interface temperature, 300 °C; DL temperature, 250 °C; and heat block temperature, 400 °C; CID gas, 230kPa. Data processing was essentially as described previously (Setoyama et al., 2016).

2.5. Statistical analysis

The statistical analyses were performed using IBM SPSS Statistics 23.0 (IBM Japan, Ltd., Tokyo, Japan). All results were expressed as mean values (mean ± standard deviation) or median values (median (interquartile range)). The normality of data was tested with Shapiro-Wilk test. To achieve normality, serotonin, indolecarboxaldehyde, mean values (mean ± standard deviation) or median values (median (interquartile range)); 26–35.1 min, 100%B; 35.1–38 min, 20%B. Parameters for positive electrospray ionization mode under multiple reaction monitoring were as follows; drying gas flow rate, 10 L/min; nebulizer gas flow rate, 3 L/min; heating gas flow rate, 10 L/min; interface temperature, 300 °C; DL temperature, 250 °C; and heat block temperature, 400 °C; CID gas, 230kPa. Data processing was essentially as described previously (Setoyama et al., 2016).

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The statistical analysis performed in the discriminant analysis with stepwise selection using diagnosis as the dependent variable, and significant differential blood biomarkers between two groups as explanatory variables (sex) between HC and MDD group. To estimate the diagnostic efficiency of blood biomarkers, we performed canonical discriminant analysis with stepwise selection using diagnosis as the dependent variable, and significant differential blood biomarkers between two groups as explanatory variables. Cross-validation procedure by leave-one-out classification was performed in the discriminant analysis with stepwise selection. Moreover, we analyzed the performance of identified blood biomarkers by receiver operating characteristic (ROC) curve. To assess the relationship between blood biomarkers and BDI-II total score, we performed Pearson's correlation analysis. In this correlation analysis, the correct classification rate obtained from discriminant analysis between HC and MDD group was 76.5%, with a sensitivity and specificity of 80.0% and 73.7%, respectively. To estimate the diagnostic performance of plasma tryptophan, the area under the curve (AUC) was calculated by ROC curve. Plasma tryptophan exhibited an AUC of 0.740 (95% confidence interval: 0.565–0.916, P = .018) (Fig. S1).

3. Results

Demographics and clinical variables were described in Table 1. First-episode drug-naive MDD patients (N = 15) were in acute episode of MDD (disease duration: 4.0 (4.0) weeks). SOD of patients was between moderate to severe in accordance with BDI-II total score (33.1 ± 12.0). There were no significant differences in sex and age between HC and MDD group.

3.1. Comparison of blood biomarker levels

Laboratory characteristics were described in Table 2 and Table S1. Plasma tryptophan and kynurenic acid were significantly lower in MDD group compared to HC group (P = .010, P = .018, respectively).

3.2. Diagnostic efficiency of blood biomarkers

Stepwise selection of the discriminant analysis between HC and MDD group was performed to estimate the diagnostic efficiency using plasma tryptophan and kynurenic acid. Plasma tryptophan was selected as the biomarker significantly associated with MDD diagnosis through the stepwise selection (P = .010). The discriminant function was significant (F = 7.533, df1 = 1, df2 = 32, P = .010), and calculated for the assessment of MDD group as follows: D = −5.650 + 0.393 (tryptophan) (HC group: D > 0, MDD group: D < 0). The correct classification rate obtained from discriminant analysis between HC and MDD group was 76.5%, with a sensitivity and specificity of 80.0% and 73.7%, respectively. To estimate the diagnostic performance of plasma tryptophan, the area under the curve (AUC) was calculated by ROC curve. Plasma tryptophan exhibited an AUC of 0.740 (95% confidence interval = 0.565–0.916, P = .018) (Fig. S1).

3.3. Relationship between blood biomarkers and SOD

As shown in Table S2, serum HDL-C was negatively correlated with BDI-II total score (r = −0.666, P = .007). Moreover, we performed single linear regression analysis to predict BDI-II total score of patients based on serum HDL-C. The significant regression equation was found (F (1, 13) = 10.381, R2 = 0.444, P = .007), as follows: predicted BDI-II total score of patients = 78.994 − 0.702 (HDL-C) (Fig. 1). Moreover, to control the above regression analysis by age and sex, we performed multiple linear regression analysis using BDI-II total score as the dependent variable, and HDL-C, age, and sex as explanatory variables with forced entry. After control by age and sex, serum HDL-C still exhibited a significant predictive ability of SOD (partial correlation coefficient = −0.619, t = −2.611, P = .024).

3.4. Relationship between blood biomarkers and depressive symptoms

As shown in Table 3 and Table S3, there was positive correlation between (1) plasma tryptophan and “motivational” symptoms such as suicidal thoughts, and (2) serum LDL-C and “vegetative” symptoms such as changes in appetite; negative correlation between (3) plasma kynurenic acid and “vegetative” symptoms, (4) plasma kynurenine/tryptophan ratio and “motivational” symptoms, and (5) serum HDL-C and various depressive sub-symptoms especially “affective” symptoms such as sadness and loss of pleasure. Moreover, as shown in Table S4, several plasma lipid species were significantly correlated with depressive sub-symptoms. In particular, plasma CE 20:0 and CE 20:1 were negatively correlated with depressive sub-symptoms such as “motivational” symptoms including suicidal thoughts.

3.5. Relationship between blood tryptophan metabolites and lipid species

As shown in Fig. 2 and Table S5, several plasma CEs including CE 20:0 and CE 20:1 were significantly correlated with plasma tryptophan and kynurenic acid in MDD group. On the other hand, the above significant correlation between plasma tryptophan-kynurenine metabolites and lipid species in MDD group was not detected in HC group (Fig. S2 and Table S6).
Table 1
Demographics and clinical variables of healthy controls and first-episode drug-naïve MDD patients.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Healthy controls</th>
<th>First-episode drug-naïve MDD patients</th>
<th>Statistics</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (Male/Female)</td>
<td>19 (10/9)</td>
<td>15 (9/6)</td>
<td>(\chi^2 = 0.185)</td>
<td>1</td>
<td>0.667</td>
</tr>
<tr>
<td>Age (year)</td>
<td>30.2 (6.8)</td>
<td>30.1 (7.6)</td>
<td>(t = 0.058)</td>
<td>32</td>
<td>0.954</td>
</tr>
<tr>
<td>Disease duration (week)</td>
<td>– (4.0)</td>
<td>– (4.0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clinical variables</td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D. or (IQR)</td>
<td></td>
</tr>
<tr>
<td>BDI-II total score</td>
<td>–</td>
<td>–</td>
<td>33.1 (12.0)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Statistical P-values were derived from chi-squared test and Student’s t-test.

Abbreviations: MDD, major depressive disorder; IQR, interquartile range.
BDI-II, Beck Depression Inventory-Second Edition.

Table 2
Comparison of blood biomarker levels between healthy controls and first-episode drug-naïve MDD patients.

<table>
<thead>
<tr>
<th>Blood biomarkers</th>
<th>Healthy controls (N = 19)</th>
<th>First-episode drug-naïve MDD patients (N = 15)</th>
<th>Statistics</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan ((\mu M))</td>
<td>15.43 (2.37)</td>
<td>13.02 (2.75)</td>
<td>(t = 2.745)</td>
<td>32</td>
<td>0.010</td>
</tr>
<tr>
<td>Indoleacetic acid ((\mu M))</td>
<td>0.107 (0.053)</td>
<td>0.111 (0.040)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log serotonin ((\mu M))</td>
<td>– 5.29 (0.37)</td>
<td>– 5.49 (0.43)</td>
<td>(t = 1.446)</td>
<td>32</td>
<td>0.158</td>
</tr>
<tr>
<td>Kynurenine ((\mu M))</td>
<td>0.127 (0.033)</td>
<td>0.097 (0.035)</td>
<td>(t = 2.498)</td>
<td>32</td>
<td>0.018</td>
</tr>
<tr>
<td>Kynurenic acid ((\mu M))</td>
<td>0.0032 (0.0016)</td>
<td>0.0030 (0.0013)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kynurenine/tryptophan ratio</td>
<td>0.0084 (0.0026)</td>
<td>0.0075 (0.0026)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total-C (mg/dL)</td>
<td>183.5 (41.7)</td>
<td>175.1 (25.8)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>66.11 (14.53)</td>
<td>65.33 (11.36)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>117.4 (43.8)</td>
<td>109.8 (24.0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log Fib (mg/dL)</td>
<td>– 5.33 (5.44)</td>
<td>– 4.54 (0.20)</td>
<td>(t = 0.515)</td>
<td>32</td>
<td>0.610</td>
</tr>
<tr>
<td>Log T-Bil (mg/dL)</td>
<td>– 0.53 (0.48)</td>
<td>– 0.56 (0.41)</td>
<td>(t = 0.236)</td>
<td>32</td>
<td>0.815</td>
</tr>
<tr>
<td>Log D-Bil (mg/dL)</td>
<td>– 1.68 (0.45)</td>
<td>– 1.71 (0.40)</td>
<td>(t = 0.245)</td>
<td>32</td>
<td>0.808</td>
</tr>
<tr>
<td>Log I-Bil (mg/dL)</td>
<td>– 0.92 (0.41)</td>
<td>– 0.95 (0.44)</td>
<td>(t = 0.199)</td>
<td>32</td>
<td>0.843</td>
</tr>
<tr>
<td>UA (mg/dL)</td>
<td>5.09 (1.20)</td>
<td>5.03 (1.10)</td>
<td>(t = 0.153)</td>
<td>32</td>
<td>0.879</td>
</tr>
<tr>
<td>Log hsCRP (ng/dL)</td>
<td>– 7.25 (0.68)</td>
<td>– 7.36 (1.16)</td>
<td>(t = 0.109)</td>
<td>32</td>
<td>0.282</td>
</tr>
</tbody>
</table>

Statistical P-values were derived from Student’s t-test.
Significant P-values are shown in bold type.

Abbreviations: MDD, major depressive disorder; Total-C, total-cholesterol.
HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; Fib, fibrinogen.
T-bil, total-bilirubin; D-bil, direct-bilirubin; I-bil, indirect-bilirubin; UA, uric acid.
hsCRP, high-sensitivity C-reactive protein.

Table 3
Significant relationship between blood biomarkers and scores of BDI-II sub-categories in first-episode drug-naïve MDD patients (N = 15).

<table>
<thead>
<tr>
<th>BDI-II sub-categories</th>
<th>Blood biomarkers</th>
<th>(\rho)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Affective” symptoms (sadness, loss of pleasure, etc.)</td>
<td>HDL-C (mg/dL)</td>
<td>– 0.843</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>“Motivational” symptoms (suicidal thoughts, etc.)</td>
<td>Tryptophan ((\mu M))</td>
<td>0.540</td>
<td>0.038</td>
</tr>
<tr>
<td>“Cognitive distortions” symptoms (guilty feelings, worthlessness, etc.)</td>
<td>Kynurenine/tryptophan ratio</td>
<td>– 0.555</td>
<td>0.032</td>
</tr>
<tr>
<td>“Behavioral” symptoms (agitation, loss of energy, etc.)</td>
<td>HDL-C (mg/dL)</td>
<td>– 0.545</td>
<td>0.036</td>
</tr>
<tr>
<td>“Vegetative” symptoms (changes in appetite, etc.)</td>
<td>Kynurenic acid ((\mu M))</td>
<td>– 0.676</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Statistical P-values were derived from Spearman’s correlation analysis.

Abbreviations: MDD, major depressive disorder; BDI-II, Beck Depression Inventory-Second Edition.
HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.
4. Discussion

This is the first blood biomarker study combining tryptophan metabolites, routine biochemical markers, and lipid species in first-episode drug-naïve MDD patients. In this exploratory pilot study, plasma tryptophan and kynurenine levels were significantly lower in MDD group compared to HC group; plasma tryptophan exhibited a moderate predictive ability of diagnosis in the discriminant analysis and ROC curve. SOD (BDI-II total score) was negatively correlated with serum HDL-C in MDD group; serum HDL-C exhibited a moderate predictive ability of SOD in linear regression analysis. In addition, severities of depressive sub-symptoms (based on scores of BDI-II sub-categories) were variously correlated with plasma tryptophan-kynurenine and lipid related metabolites. Moreover, plasma tryptophan-kynurenine metabolites and CE were significantly correlated in MDD group, but not in HC group. We have summarized the present findings in Fig. 3.

Supporting the present results, a meta-analysis study has shown reduced plasma tryptophan levels in patients with MDD, particularly in unmedicated patients (Ogawa et al., 2014). Clinical studies measuring plasma tryptophan levels in first-episode drug-naïve MDD patients are limited, while some previous studies have also shown reduced plasma tryptophan levels in first-episode drug-naïve MDD patients (Liu et al., 2015b; Xu et al., 2012). Recent evidence including our present data has offered a potential utility for measuring plasma tryptophan as an objective tool for supporting diagnosis of MDD patients in clinical practice, especially for early intervention.

The “serotonin hypothesis” detailing serotonin-system dysfunction in the brain has been a central and longstanding hypothesis of depression (Coppen, 1967; Fava and Kendler, 2000; Oxenkrug, 2013). Serotonin synthesis in the brain is considerably dependent on the availability to deliver an adequate volume of tryptophan, a precursor for serotonin, from blood to the brain (Fernstrom, 1977). Lower volume of serotonin in the brain after tryptophan depletion has been suggested to lead to depressive symptoms (Young, 2013; Young et al., 1985).
Several studies have shown altered regulation of serotonin-2 receptors in response to tryptophan depletion (Price et al., 1997; Yatham et al., 2012). In addition, there has been some evidence showing the effect of tryptophan loading as a treatment for depression (Parker and Brotchie, 2011). The present results and recent evidence suggest that tryptophan supplementation may improve patients with depression.

In the reconstruction of the conventional serotonin hypothesis of depression, a secondary metabolic pathway of tryptophan, called tryptophan-kynurenine pathway, has recently attracted attention (Dantzer, 2017; Dantzer et al., 2008; Dantzer et al., 2011; Halaris, 2013, 2017; Maes et al., 2012; Maes et al., 2011b; Maes et al., 2009; Myint, 2012; Myint et al., 2007; Myint et al., 2012; Ormsstad et al., 2016; Roomruangwong et al., 2017; Schwarcz et al., 2012; Schwarz and Stone, 2017). Lower kynurenic acid, a kynurenine-pathway metabolite, is suggested to lead to lower neuroprotective activity, since kynurenic acid is an antagonist of N-methyl-D-aspartate receptors and alpha7-nicotinylcholine receptors (Dantzer et al., 2011; Maes et al., 2011b; Myint, 2012; Myint et al., 2012; Schwarz et al., 2012; Schwarz and Stone, 2017). In the present study, we have shown positive correlation between plasma tryptophan and “motivational” symptoms including suicidal thoughts, and negative correlation between plasma kynurenic acid and “vegetative” symptoms. The activation of TDO and IDO, which degrades tryptophan to kynurenine along this pathway, is suggested to reduce serotonin production through upregulation of tryptophan degradation, and such imbalance of tryptophan-kynurenine pathway is suggested to be involved in the development of depression (Dantzer et al., 2011; Halaris et al., 2015; Maes et al., 2012; Maes et al., 2011b; Myint, 2012; Myint et al., 2012). In the present study, there has been no significant difference in the plasma kynurenine/tryptophan ratio, an indicator for the activity of TDO and IDO, between HC and MDD group. However, we have shown that plasma kynurenine/tryptophan ratio is negatively correlated with “motivational” symptoms including suicidal thoughts. On the other hand, a previous study has shown that plasma tryptophan levels and activity of TDO and IDO are elevated in MDD patients with a history of suicide attempts (Sublette et al., 2011). Several previous reports have suggested the upregulation of tryptophan-kynurenine pathway in suicidality, while other reports have suggested contradictory findings (Bay-Richter et al., 2015; Brundin et al., 2016; Busse et al., 2015; Setoyama et al., 2016). Further clinical studies especially longitudinal studies should be conducted to clarify how tryptophan-kynurenine pathway contributes to the underlying mechanisms of suicidality. Substances not measured in the present study are neurotoxic kynurenine-pathway metabolites such as 3-hydroxykynurenine and quinolinic acid that have been suggested to be important for further understanding of imbalance of tryptophan-kynurenine pathway in MDD (Dantzer et al., 2011; Halaris et al., 2015; Steiner et al., 2011). In addition, inflammation is deeply involved with both MDD and tryptophan-kynurenine pathway, while we did not measure inflammatory markers except for hsCRP. Proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are known to activate IDO, as a result, inhibit biosynthesis from tryptophan to serotonin (Dantzer et al., 2011; Halaris et al., 2015; Maes et al., 2012; Maes et al., 2011b; Myint, 2012; Myint et al., 2012). Moreover, many studies have reported that brain inflammation through proinflammatory cytokines, etc., plays an important role in the pathophysiology of MDD, and that enhanced neurodegeneration in MDD may be caused by inflammatory processes (Dantzer et al., 2008; Goldsmith et al., 2016; Halaris, 2013, 2017; Maes et al., 2009). Further studies combining inflammatory markers, such as IL-6 and TNF-α, and tryptophan-kynurenine metabolites including 3-hydroxykynurenine and quinolinic acid should be conducted to reveal the underlying relationships between MDD, inflammation, and tryptophan-kynurenine pathway.

Blood cholesterol such as HDL-C and LDL-C have been suggested to be involved in the pathophysiology of MDD (Parekh et al., 2017; Shelton and Miller, 2010). To our knowledge, this is the first study to suggest that serum HDL-C is negatively correlated with SOD in first-episode drug-naïve MDD patients. Based on the present results, we have proposed lower serum HDL-C as a predictive blood biomarker of SOD especially for first-episode drug-naïve MDD patients. Supporting the present study, several studies have reported that serum HDL-C is negatively correlated with SOD in patients with MDD (Lucking et al., 2017; Shin et al., 2008). HDL-C is known to be carried by a lipoprotein, HDL (Camont et al., 2011). HDL possesses antioxidative properties by protecting LDL from oxidative damage induced by free radicals, etc., and through removal of oxidised lipids from oxidised LDL with subsequent inactivation (Camont et al., 2011). Moreover, HDL also inhibits intracellular generation of reactive oxygen species (Camont et al., 2011). Therefore, HDL-C levels may be expressive of HDL antioxidative activities (Liu et al., 2015a). On the other hand, oxidative stress is known to cause cellular damage such as lipid peroxidation, enzyme inactivation, and DNA modification (Fridovich, 1986). Moreover, oxidative stress is suggested to disrupt the blood-brain-barrier, allowing neuro-pathological species such as proinflammatory cytokines to enter the brain (Halliwell, 2006; Najar et al., 2013). Many studies have shown elevated oxidative stress in patients with MDD (Herken et al., 2007; Liu et al., 2015a; Maes et al., 2010), thus oxidative stress is regarded to be involved in the pathophysiology of MDD (de Melo et al., 2017; Maes et al., 2011a; Michel et al., 2012). Interestingly, the antidepressant effect of omega-3 fatty acids is suggested to be partly associated with improvement of HDL function relevant to the upregulation of antioxidative activity (Burlillo et al., 2012; Grosso et al., 2014). Thus, we have hypothesized that the downregulation of antioxidative activity due to lower serum HDL-C levels may lead to increased SOD, and that HDL-C may work as an antidepressant through antioxidative activity.

The present metabolome/lipidome analysis has revealed that plasma lipid species such as LPCs, PCs, PE’s, CEs, and TGs are variously correlated with several depressive sub-symptoms. These lipid species provide important biological functions such as membrane structure, energy storage, and signal transduction, and act as a cellular mediator (Fonteh et al., 2006; Liu et al., 2016; Liu et al., 2015b). Therefore, lipid species have been suggested to enhance existing knowledge of brain diseases including MDD (Fonteh et al., 2006; Liu et al., 2016; Liu et al., 2015b). Interestingly, the present study has shown that some plasma CEs are negatively correlated with “motivational” symptoms including suicidal thoughts. Supporting the present results, meta-analysis studies have suggested that serum cholesterol is negatively associated with suicidality (Muldoon et al., 1990; Wu et al., 2016). CEs, formed by the esterification of cholesterol, play an important part in cholesterol transportation and homeostasis (Tosi and Tugnoli, 2005). Through esterification of cholesterol, blood cholesterol is transported in blood by lipoproteins such as HDL and LDL, and can be accumulated and utilized in cells (Camont et al., 2011; Tosi and Tugnoli, 2005). Moreover, cholesterol serves multiple important functions such as maintenance of plasma membrane fluidity, neurogenesis, and transmembrane protein and receptor activity (Fonteh et al., 2006). Therefore, changes in cholesterol metabolism significantly modulate brain function (Fonteh et al., 2006). Based on the above, we propose that lower esterification in cholesterol may constitute the pathophysiology of MDD through altered plasma membrane fluidity and receptors function such as serotonin receptor (Heron et al., 1980; Maes et al., 1994).

How peripheral cholesterol influences brain systems is unclear, due to the inability of cholesterol to cross the blood–brain-barrier (Parekh et al., 2017). On the other hand, a recent clinical study has shown that cholesterol-lowering treatment leads to downregulation of tryptophan-kynurenine pathway parallelly by oxidative stress improvement in patients with chronic kidney disease (Zinelli et al., 2015). Moreover, a recent rodent study has shown that serum lipid species are involved with altered function of tryptophan metabolites (Weng et al., 2016). Thus, we have hypothesized that other than the above-discussed relationship between HDL-C and oxidative stress, the relationship between peripheral tryptophan metabolites and CEs may explain how
peripheral cholesterol effects brain activities. Supporting our hypothesis, the present study has shown that plasma tryptophan levels are lower and plasma kynurenic acid levels are higher during higher plasma CEs levels in patients with MDD, but not in HC. In addition, the present study has shown that such alteration of the above substances is significantly associated with lower depressive symptoms (Fig. 3). While peripheral kynurenic acid mostly does not cross the blood-brain-barrier (Nemeth et al., 2005), peripheral kynurenic acid may be involved in the pathophysiology of MDD through its antioxidative activity on reactive oxygen species (Lugo-Huitron et al., 2011). Therefore, plasma CEs may link with brain systems through the antioxidative activity of plasma kynurenic acid, which may be involved in the pathophysiology of MDD. Both tryptophan-kynurenine metabolites and cholesterol have been suggested to be involved in inflammation (Dantzer, 2017; Dantzer et al., 2011; Halaris et al., 2015; Maes et al., 2012; Maes et al., 2011b; Myint, 2012; Myint et al., 2012; Parekh et al., 2017). Brain inflammation induced by microglial activation is now highlighted in various psychiatric disorders including MDD (Hayakawa et al., 2014; Horikawa et al., 2010; Kato et al., 2011a; Kato et al., 2013; Monji et al., 2009; Ogidani et al., 2016; Sato-Kasai et al., 2016). Further studies are needed to clarify the interactions between tryptophan-kynurenine metabolites, cholesterol, CEs, inflammation, and microglial activation by utilizing cross-species studies with animal models of depression and patients with depression.

4.1. Limitations

The present exploratory pilot study has some limitations. First, the main limitation is sample size. Small sample size has its restriction in drawing conclusive findings, and the present findings are preliminary. In addition, we did not use multiple test correction to avoid the risk of false negatives, as the purpose of this study was the global analysis of a number of blood biomarkers as an exploratory pilot study for a future validation study. Despite the small sample size, we have successfully detected several statistically significant data. Since candidate biomarkers have been identified, follow-up studies with greater sample size should be conducted to validate our preliminary findings. Machine learning is known to be a useful technique to evaluate the validity of the results in biomarker studies (Angermueller et al., 2016). Thus, in the future validation studies, machine learning should be conducted to improve the quality of research. On the other hand, low statistical power due to small sample size of the present study led to the lack of significance in many substances. One of these substances is CRP with accumulated evidence that CRP is higher in patients with MDD (Haapakoski et al., 2015; Howren et al., 2009). In addition, Maes and his colleagues have investigated the action of xanthurenic acid, one of the final metabolites in tryptophan-kynurenine pathway, and have suggested that xanthurenic acid is involved with psychiatric disorders including depression through an anti-inflammatory effect (Kanchanatawan et al., 2017; Maes et al., 1986; Maes et al., 2007). However, as mentioned above, the present findings are preliminary, since drawing conclusive findings is difficult due to small sample size. In addition, the levels of some blood biomarkers may change according to the stage of MDD. Further longitudinal studies with greater sample size should be conducted. Second, the disease specificity of biomarkers is a critical issue in the present study. Tryptophan, kynurenine metabolites, and CEs have been reported to be involved not only with MDD but also with various psychiatric disorders such as schizophrenia and bipolar disorder and physical disorders such as inflammatory bowel diseases, infectious diseases, and heart diseases (Choi et al., 2017; Dantzer et al., 2011; Halaris, 2013, 2017; Maes et al., 2011b; Martin-Subero et al., 2016; Murakami et al., 2013; Myint, 2012; Schwarz et al., 2012; Sorel-Thomas and Thomas, 2016). Interestingly, depression, or at least some depressive symptoms, also occur in the above disorders. Therefore, the candidate biomarkers in the present study may be common biomarkers for depressive symptoms in various psychiatric and physical disorders. An integrative approach towards depression in various diseases is warranted to search for common biomarkers for depressive symptoms beyond the boundaries of certain diseases. Third, we did not assess body mass index (BMI) and diet conditions. Levels of some blood biomarkers are affected by BMI and diet. Further studies should check BMI and as to whether blood samples were collected under fasting or non-fasting conditions. Fourth, in the exclusion criteria of HC, we did not check psychiatric and physical disorders in first-degree relatives. Levels of some blood biomarkers may be affected by family history, especially by family history of diseases with genetic predisposition. Further studies should check psychiatric and physical disorders of the first degree relatives of HC.

4.2. Conclusion

In the present study, we have shown the first findings that not only tryptophan-kynurenine metabolites but also HDL-C and CEs are important blood biomarkers for first-episode drug-naïve MDD patients. The present study has shed new light on the role of tryptophan-kynurenine and lipid related metabolites in the pathophysiology of MDD. The diagnosis and symptom evaluation of MDD in clinical practice has depended on physician’s clinical experiences and patient’s self-assessments. As a result, there are many delayed consultations and treatments, and misdiagnoses. The blood biomarkers proposed in the present study are possible objective diagnostic indicators for first-episode drug-naïve patients with depression, which are particularly important and useful for early detection not only in psychiatric settings but also primary care and corporate health settings. The present findings are expected to be validated by larger population studies combining with machine learning technique. In addition, further studies should evaluate whether the present findings can be also applied to other situations, such as prognosis evaluation and patients under medication.

Acknowledgments

The authors would like to thank Dr. Koji Tanaka, Mr. Keita Kurahara, Ms. Ryoko Katsuki, and Ms. Sakumi Kakimoto for their research support and/or assistance.

Role of funding source

This work was partially supported by Grant-in-Aid for Scientific Research on (1) Innovative Areas “Will-Dynamics” and “Glia Assembly” of The Ministry of Education, Culture, Sports, Science, and Technology, Japan (JP16H06403 to T.A.K.; JP25117011 to S.K.), (2) The Japan Agency for Medical Research and Development (AMED) (Syogaisya-Taisaku-Sogo-Kenkyu-Kaihatsu-Jigyo to T.A.K. & S.K. (16dk0307047h0002), and Yugo-no to T.A.K. (16dm0107095h0001)), (3) KAKENHI - the Japan Society for the Promotion of Science (JP26713039, JP15K15431, & JP16H03741 to T.A.K., and JP16H02666 to S.K.), and (4) SENSIN Medical Research Foundation (to T.A.K. and S.K.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2018.01.014.

References


