Metabolomic Study on Fatty Acids in Placenta of Preeclamptic Pregnancies by Gas Chromatography-Mass Spectrometry

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Abstract

Preeclampsia is a serious pregnancy-associated complication and can critically affect the health of the mother and fetus. However, the pathogenesis of preeclampsia remains unclear. One of the characteristics of preeclampsia is abnormal lipid metabolism. Thus, profiling analysis of 23 fatty acids (FAs) as tert-butyldimethylsilyl derivative was performed in the placenta of preeclamptic term pregnancies and uncomplicated term pregnancies by gas chromatography-mass spectrometry. The compositions of saturated, monounsaturated and n-3 polyunsaturated FAs were significantly reduced, whereas those of oleic acid among monounsaturated FAs and arachidonic acid among n-6 polyunsaturated FAs were significantly increased in preeclampsia group compared to the normal group. The distorted star pattern of the preeclamptic pregnancy group was different from the tricosagonal shape of the normal group. Thus, the present FA profiling analysis combined with the star symbol plotting method will be useful for the biochemical monitoring of placental abnormalities and visual discrimination between preeclamptic and normal pregnancies.

Keywords: Fatty acid profiling analysis; Star symbol plotting; Preeclampsia; Placenta; GC-MS

Introduction

Preeclampsia, a disorder that occurs in about 5% of pregnant women, is the main cause of poor perinatal outcome affecting both the mother and fetus. The clinical features are characterized by maternal hypertension, proteinuria, and edema [1,2]. However, the cause of preeclampsia is unclear, and this condition has become an important issue. The pathogenic explanations include oxidative stress, increased inflammatory reaction, and dyslipidemia [3]. The exaggerated lipid adaptation of preeclamptic pregnancy, including free fatty acid (FFA), is a very important mechanism in preeclampsia, which shows close relationships with oxidative stress [4].

Previous studies of altered polyunsaturated fatty acid (PUFA) suggested that increased inflammation and oxidative stress in maternal blood and placental tissues of preeclampsia, compared to normal pregnancies [5]. However, simultaneous studies of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and PUFA have not been performed in preeclamptic pregnancies. Thus, simultaneous metabolic profiling analysis of SFA, MUFA, and PUFA as tert-butyldimethylsilyl...
derivative in placental tissue samples from normal and preeclamptic pregnant women was performed for monitoring of altered FA metabolic pattern by gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode.

The accurate discrimination between normal and abnormal states requires the application of an appropriate computer-aided pattern recognition method to complex metabolic profiles. In our previous studies, star symbol plotting as a visual pattern recognition method readily allowed discrimination between patients with X-linked adrenoleukodystrophy andnormal controls for comparative analysis of very long chain FAs in plasma [6]. The same approach was also applied to FFA profiling analysis in the plasma of rats with viral infection, which was useful for the comparative analysis of FFA profiles between two viral infection groups and control [7]. And FFA profiling analysis in plasma and brain was useful for monitoring of altered FFA metabolism following cell therapy with human bone marrow-derived mesenchymal stem cell in ischemia rat model [6].

Therefore, in this study, the metabolomic analysis of FAs combined with star pattern recognition analysis in placental tissue samples from normal and preeclamptic pregnancy groups was performed for biochemical monitoring of altered FA metabolic patterns.

Materials and Methods

Chemicals and reagents

The 24 FA standards, including pentadecanoic acid as an internal standard (IS) and triethylamine (TEA), were purchased from Sigma (St. Louis, MO). N-Methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL). Acetonitrile, toluene, diethyl ether, and dichloromethane (pesticide grade) were obtained from Duksan (Seoul, South Korea).

Gas chromatography-mass spectrometry

GC-MS analysis in SIM mode for quantitative analysis of FFA in the placental tissues was performed with an Agilent 6890 gas chromatograph, interfaced to an Agilent 5973 mass-selective detector (70 eV, electron impact mode) with an Ultra-2 (SE-54 bonded phase; 25 m × 0.20 mm I.D., 0.11 µm film thickness) cross-linked capillary column (Agilent Technologies, Atlanta, GA). Helium was used as the carrier gas at a flow rate of 0.5 mL/min in constant flow mode. The injector, interface, and ion source were maintained at 260, 300, and 230°C, respectively. Samples were introduced in the split-injection mode (10:1). The oven temperature was set initially at 100°C (2 min) and then programmed at a rate of 4°C/min to 260°C, and finally at 20°C/min to 300°C (10 min). The scanned mass range was 50-750 u at a rate of 0.99 scan/s.

Sample preparation for assaying FFAs in placental tissue samples

The study was approved by the Ethics Committee of Ajou University Medical Center (project No. CRO106). The participants and their family members were fully informed about this study before enrollment and they signed written consent forms. Five women with severe preeclampsia without intrauterine growth restriction, matched by term gestational age at the time of delivery, maternal age, obstetric history, and pre-pregnant BMI with five normal pregnant women were enrolled in this study. All subjects received regular antenatal care of which institution and were administered identical prenatal vitamins from the twentieth gestational weeks without additional omega-3 supplement. All subjects showed similar income, education levels, lifestyle, and food intake through questionnaire.

Sample preparation for FFA profiling analysis in the placental tissue samples from normal (n=5) and preeclamptic (n=5) pregnant women was performed according to our previously described method [7-9]. Placental tissues were homogenized (3 min, 30,000 rpm) in 5 mL of distilled water in an ice-water bath using a rotor/stator-type tissue homogenizer (Model Pro 200 Homogenizer; Pro Scientific, Monroe, CT). An aliquot (equivalent to 20 mg of placental tissue) including pentadecanoic acid (5.0 µg) as the IS was vortex-mixed with acetonitrile (1 mL) for 3 min. The mixture was centrifuged at 15,000 rpm (15 min) for protein precipitation. Briefly, 1 mL of distilled water was added to the supernatant after centrifugation. Aliquots were then adjusted to pH ≥ 12 with 5.0 M sodium hydroxide and washed with diethyl ether (3 mL × 2). The aqueous phase was then acidified to pH ≤ 2.0 with concentrated sulfuric acid and saturated with sodium chloride, followed by extraction with diethyl ether (3 mL × 2). The extracts were evaporated to dryness under a gentle stream of nitrogen gas. The dry residues containing FFAs were reacted (60°C for 30 min) with TEA (5 µL), toluene (20 µL), and MTBSTFA (20 µL) to form TBDMS derivatives. All samples were analyzed in triplicate and examined directly by GC-MS with SIM mode.

Pattern recognition analysis

The FFA levels were summated after calculation from the calibration curves and the compositions of each FA in the total FFAs were expressed as percentages (%). The FFA values of each sample were normalized.
to the corresponding mean in the normal group. Subsequently, each normalized value was plotted as a line radiating from a common central point, and the far ends of the 23 lines were joined together to produce a star pattern for each patient using Microsoft Excel® as described previously [6-8].

Results and Discussion

Clinical characteristics

There were no significant differences between the control and preeclamptic groups in maternal age, obstetric history, pre-pregnant BMI, gestational age at delivery, and neonatal birth weight. The BMI at the time of delivery, maternal systolic blood pressure, maternal diastolic blood pressure, maternal hemoglobin (g/dL) levels and hematocrit were significantly higher in the preeclamptic group than the control group (P<0.05, Table 1).

FFA compositions in placental tissues of normal and preeclamptic pregnancy groups

The compositions (%) of 23 FFA levels (ng/mg) in placental tissues from normal and preeclamptic pregnancies. Table 2:

| No. | Fatty acid (C<sub>n</sub>:<sub>m</sub>) | Normal (n=5) | Preeclampsia (n=5) | P value<sup>a</sup> |
|-----|-----------------------------------|-------------|-------------------|----------------|
| 1   | Decenolic acid (C<sub>10:1</sub>)  | 0.16 ± 0.02 | 0.10              | 0.14           | 0.15 ± 0.11 | 0.14 | 0.13 ± 0.02 | 0.04 |
| 2   | Capric acid (C<sub>10:0</sub>)    | 0.49 ± 0.04 | 0.34              | 0.44            | 0.48 ± 0.36 | 0.47 | 0.42 ± 0.06 | 0.03 |
| 3   | Lauric acid (C<sub>12:0</sub>)    | 0.57 ± 0.07 | 0.36              | 0.50            | 0.53 ± 0.41 | 0.51 | 0.46 ± 0.07 | 0.02 |
| 4   | Myristoleic acid (C<sub>14:1</sub>) | 1.27 ± 0.15 | 0.78              | 1.14            | 1.24 ± 0.93 | 1.18 | 1.05 ± 0.19 | 0.04 |
| 5   | Myristic acid (C<sub>14:0</sub>)   | 0.82 ± 0.08 | 0.55              | 0.74            | 0.81 ± 0.64 | 0.77 | 0.70 ± 0.11 | 0.03 |
| 6   | Palmitoleic acid (C<sub>16:1</sub>) | 4.59 ± 0.19 | 4.05              | 4.62            | 5.14 ± 0.61 | 4.53 | 0.47 ± 0.70 | 0.3  |
| 7   | Palmitic acid (C<sub>16:0</sub>)   | 8.16 ± 1.48 | 11.13             | 9.29            | 7.25 ± 12.48 | 9.64 | 9.96 ± 1.97 | 0.07 |
| 8   | γ-Linolenic acid (C<sub>18:3n3</sub>) | 4.08 ± 0.17 | 2.52              | 3.65            | 4.00 ± 2.99 | 3.78 | 3.39 ± 0.61 | 0.04 |
| 9   | Linoleic acid (C<sub>18:2n6</sub>) | 3.32 ± 0.41 | 3.27              | 2.96            | 3.18 ± 2.94 | 3.12 | 3.09 ± 0.14 | 0.1  |
| 10  | Oleic acid (C<sub>18:1</sub>)      | 19.03 ± 2.32 | 25.34             | 20.86           | 19.95 ± 22.54 | 19.95 | 21.73 ± 2.28 | 0.05 |
| 11  | α-Linolenic acid (C<sub>18:3n3</sub>) | 0.82 ± 0.08 | 0.53              | 0.73            | 0.80 ± 0.63 | 0.75 | 0.69 ± 0.11 | 0.03 |
| 12  | Stearic acid (C<sub>18:0</sub>)    | 6.06 ± 0.71 | 8.31              | 6.84            | 4.33 ± 7.67 | 6.60 | 6.75 ± 1.51 | 0.2  |
| 13  | Arachidonic acid (C<sub>20:4n6</sub>) | 10.44 ± 1.23 | 16.13             | 11.35           | 11.35 ± 12.53 | 11.55 | 12.70 ± 1.98 | 0.03 |
| 14  | Eicosa-pentaenoic acid (C<sub>20:5n3</sub>) | 4.65 ± 0.45 | 3.39              | 4.34            | 4.90 ± 3.58 | 4.30 | 4.10 ± 0.61 | 0.07 |
| 15  | Eicosenoic acid (C<sub>20:0</sub>)  | 4.17 ± 0.47 | 2.67              | 3.77            | 4.09 ± 3.10 | 3.89 | 3.50 ± 0.60 | 0.04 |
| 16  | Eicosanoic acid (C<sub>20:4</sub>)  | 3.04 ± 0.35 | 1.89              | 2.73            | 2.97 ± 2.24 | 2.82 | 2.53 ± 0.45 | 0.04 |
| 17  | Docosahexaenoic acid (C<sub>22:6n3</sub>) | 7.58 ± 0.44 | 5.66              | 7.22            | 7.87 ± 5.96 | 6.74 | 6.69 ± 0.90 | 0.04 |
| 18  | Docosapentaenoic acid (C<sub>22:5n3</sub>) | 4.36 ± 0.48 | 2.85              | 3.96            | 4.33 ± 3.25 | 4.04 | 3.69 ± 0.61 | 0.05 |
| 19  | Erucreic acid (C<sub>22:1</sub>)   | 4.07 ± 0.47 | 2.51              | 3.65            | 3.99 ± 2.98 | 3.78 | 3.38 ± 0.61 | 0.04 |
| 20  | Behenic acid (C<sub>22:0</sub>)    | 4.40 ± 0.50 | 2.72              | 3.95            | 4.31 ± 3.23 | 4.08 | 3.66 ± 0.66 | 0.04 |
| 21  | Nervonic acid (C<sub>24:1</sub>)   | 0.24 ± 0.02 | 0.17              | 0.23            | 0.23 ± 0.18 | 0.23 | 0.21 ± 0.03 | 0.06 |
| 22  | Lignoceric acid (C<sub>24:0</sub>) | 3.84 ± 0.44 | 2.38              | 3.46            | 3.77 ± 2.82 | 3.56 | 3.20 ± 0.58 | 0.04 |
| 23  | Hexacosanoic acid (C<sub>26:0</sub>) | 3.83 ± 0.44 | 2.36              | 3.43            | 3.75 ± 2.81 | 3.55 | 3.18 ± 0.58 | 0.04 |

Total saturated fatty acids | 31.23 ± 1.31 | 30.04 | 31.38 | 28.20 | 32.66 | 32.00 | 30.86 ± 1.77 | 0.4  |
Total unsaturated fatty acids | 68.77 ± 1.31 | 69.98 | 68.62 | 71.80 | 67.33 | 67.98 | 69.14 ± 1.77 | 0.4  |
Total monounsaturated fatty acids | 33.53 ± 1.52 | 35.62 | 34.41 | 34.79 | 35.45 | 33.70 | 34.80 ± 0.79 | 0.07 |
Total polysaturated fatty acids | 35.24 ± 0.82 | 34.36 | 34.21 | 37.01 | 31.88 | 34.28 | 34.35 ± 1.81 | 0.2  |
Total n-3 polysaturated fatty acids | 17.41 ± 1.41 | 12.43 | 16.25 | 17.9 | 13.42 | 15.83 | 15.17 ± 2.21 | 0.05 |
Total n-6 polysaturated fatty acids | 17.83 ± 0.81 | 21.93 | 17.96 | 19.11 | 18.46 | 18.45 | 19.18 ± 1.59 | 0.06 |
Total n-3 / Total n-6 | 9.86 ± 0.13 | 0.57 | 0.90 | 0.73 | 0.86 | 0.80 | 0.80 ± 0.15 | 0.04 |

Table 2: FFA composition in placental tissues from normal and preeclamptic pregnancies.
The levels of each of the 23 FFAs in the preeclamptic pregnancy group were normalized to the corresponding normal mean values (Table 3). When these normalized values were used as variables to draw star graphs composed of 23 rays, star patterns (P-1 through P-5) of the 5 patients were deformed tricosagons in contrast to the small equilateral tricosagon of the normal group average placed in each center (Figure 1). In addition, the differences in mean values between the normal and preeclamptic pregnancy groups were more clearly represented in the visual star pattern for the placental tissue (Figure 1).

The tricosagonal shape of normalized FFA values in placental tissue was very informative, which expressed the elevation of FFA levels in preeclampsia. The elevation of FFA levels in placental tissue of preeclamptic pregnant women was much higher than that in the normal control group. In addition, in the preeclamptic pregnancy group, palmitic acid (No. 7) was the most abundant, followed by arachidonic acid (No. 13). Thus, the two groups could be distinguished from each other. On visual comparison of the two groups, the normal control group served well as the control pattern for the preeclamptic pregnancy group. Star symbol plots drawn based on these 23 variables were very useful for the visual pattern recognition of each group. The mean star plots representing the preeclamptic pregnancy and control groups were clearly distinguishable.

In this study, the significant reductions in n-3 PUFAs, SFAs, and MUFAs, and significant increases of arachidonic acid among n-6 PUFAs were associated with increased inflammation in preeclampsia. These findings may explain the placental abnormalities associated with altered FA metabolic patterns in preeclampsia. In addition, star plots representing preeclamptic and normal pregnancy groups were clearly distinguishable from each other.

**Conclusion**

### Table 3: Normalized values of FFA variables measured in placental tissues of five preeclamptic pregnancies.

| No. | Fatty acid                  | P-1     | P-2     | P-3     | P-4     | P-5     | Average |
|-----|-----------------------------|---------|---------|---------|---------|---------|---------|
| 1   | Decenoic acid (C20:1n9)     | 0.62    | 0.89    | 0.98    | 0.73    | 0.93    | 0.83    |
| 2   | Capric acid (C10:0)         | 0.68    | 0.90    | 0.97    | 0.73    | 0.95    | 0.85    |
| 3   | Lauric acid (C12:0)         | 0.62    | 0.87    | 0.92    | 0.72    | 0.90    | 0.81    |
| 4   | Myristoleic acid (C14:0)    | 0.62    | 0.90    | 0.98    | 0.73    | 0.93    | 0.83    |
| 5   | Myristic acid (C14:0)       | 0.66    | 0.90    | 0.98    | 0.78    | 0.93    | 0.85    |
| 6   | Palmitoleic acid (C16:1)    | 0.88    | 1.01    | 1.12    | 1.22    | 0.99    | 1.04    |
| 7   | Palmitic acid (C16:0)       | 1.36    | 1.14    | 0.89    | 1.53    | 1.18    | 1.22    |
| 8   | γ-Linolenic acid (C18:3n3)  | 0.62    | 0.90    | 0.98    | 0.73    | 0.93    | 0.83    |
| 9   | Linoleic acid (C18:2n6)     | 0.98    | 0.89    | 0.96    | 0.89    | 0.94    | 0.93    |
| 10  | Oleic acid (C18:1)          | 1.33    | 1.10    | 1.05    | 1.18    | 1.05    | 1.14    |
| 11  | α-Linolenic acid (C18:3n3)  | 0.64    | 0.88    | 0.97    | 0.76    | 0.92    | 0.83    |
| 12  | Stearic acid (C18:0)        | 1.37    | 1.13    | 0.71    | 1.27    | 1.09    | 1.11    |
| 13  | Arachidonic acid (C20:4n6)  | 1.55    | 1.09    | 1.14    | 1.20    | 1.11    | 1.22    |
| 14  | Eicosapentaenoic acid (C20:5n3) | 0.73    | 0.93    | 1.05    | 0.77    | 0.92    | 0.88    |
| 15  | Eicosenoic acid (C20:1n9)   | 0.64    | 0.90    | 0.98    | 0.74    | 0.93    | 0.84    |
| 16  | Eicosanoic acid (C20:1n9)   | 0.62    | 0.90    | 0.98    | 0.74    | 0.93    | 0.83    |
| 17  | Docosahexaenoic acid (C22:6n3) | 0.75    | 0.95    | 1.04    | 0.79    | 0.89    | 0.88    |
| 18  | Docosapentaenoic acid (C22:5n3) | 0.65    | 0.91    | 0.99    | 0.75    | 0.93    | 0.85    |
| 19  | Erucic acid (C22:1)         | 0.62    | 0.90    | 0.98    | 0.73    | 0.93    | 0.83    |
| 20  | Behenic acid (C22:0)        | 0.62    | 0.90    | 0.98    | 0.73    | 0.93    | 0.83    |
| 21  | Nervonic acid (C24:1)       | 0.71    | 0.98    | 0.98    | 0.76    | 0.95    | 0.88    |
| 22  | Lignoceric acid (C24:0)     | 0.62    | 0.90    | 0.98    | 0.73    | 0.93    | 0.83    |
| 23  | Hexacosanoic acid (C26:0)   | 0.62    | 0.90    | 0.98    | 0.73    | 0.93    | 0.83    |

*Values normalized to corresponding normal mean values*
The present metabolomic analysis of FAs combined with star symbol plotting will be useful for biochemical monitoring of altered FA metabolism in placental tissue from preeclamptic pregnancies.

Acknowledgements
This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2015R1A4A1041219) and by Suncheon Research Center for Natural Medicines.

Conflict of interest
The authors declare that they have no competing financial interests.

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