**Additional files:**

Additional file 1: Methods for Plasma TMAO concentration assay

Additional file 2: Table S1. Comparison on maternal plasma TAMOconcentration between preeclampsia and control groups

**Additional file 1**

**Methods for Plasma TMAO concentration assay**

The frozen plasma were thawed and centrifuged at 5,500×g and 4°C for 5 min. Then 100μL plasma samples were added with 300μL internal standard solution which contained a mixture of 10μmol/L of d9-TMAO prepared in methanol/ acetonitrile (15:85) and 0.2% formic acid. The mixture was briefly vortex mixed and centrifuged at 10,000 ×g, 4◦C for 5 min. The supernatant was transferred to another tube and evaporated to dryness using a vacuum drying concentrator at 25◦C. Finally, the drying residue was dissolved in100 μL of methanol-acetonitrile (25:75, v: v), followed by vortex-mixed for 2 min, and then centrifuged at 10,000 ×g for 5 min. The corresponding supernatant was transferred to an auto-sampler vial with an insert (LVI, 150μL, Waters) and 20μL was injected into the UHPLC–MS/MS system for analysis. UHPLC–MS/MS analyses were carried out using an UPLC Acquity coupled to a MicroMass Quattro Premier XE tandem quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA). Chromatographic separation was performed using an Acquity UPLC BEH HILIC column (100mm×2.1 mm; 1.7μm Waters Corporation, Milford, MA, USA). The column temperature was set to 30°C, and the flow rate was set as 0.4 mL/min and composed of Water (A) (containing 15mmol/L ammonium formate, pH = 3.5) and acetonitrile (B) as the mobile phase. The condition of isocratic elution was set to 60% B and the total running time was 3 min. All the samples were kept in the auto-sampler at 10◦C. TMAO and d9-TMAO were monitored in positive-ion mode with multiple reaction monitoring of precursor and characteristic production transitions of m/z 76.3→58.4 and 85.1→66.3, respectively. Various concentrations of non-isotopically labeled TMAO were mixed with a fixed amount of internal standard d9-TMAO to prepare the calibration curves for quantification of plasma TMAO. For quality assurance, 8 different quality-control samples with TMAO concentrations ranging between 2 and 500ng/mL were used for the evaluation of accuracy and precision. Any sample with a TMAO concentration exceeding 500ng/mL was diluted and the final concentration was calculated with use of appropriate dilution factor. The accuracy of quality-control samples was within the range of 85-105% of the nominal values, the intra- and inter assay coefficient of variations (CVs) were all below 6%, and the absolute recovery was between 85% and 106%. All of the assays were performed without knowledge of PE status.

**Additional file 2**

Table S1. Comparison on maternal plasma TAMOconcentration between preeclampsia and control groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | | T2 plasma TMAO  (median (Q1, Q3), µg/m3) | TD plasma TMAO (median (Q1, Q3), µg/m3) | Change of TMAO  (median (Q1, Q3), µg/m3) |
| Control (N=198) | | 84.05(60.44, 113.90) | 134.96 (78.10, 202.15) | 53.71( -2.60, 115.52) |
| PE (N=66) | | 92.40(53.80, 126.36) | 151.01(103.45, 280.48) | 57.47 (8.32, 211.50) |
|  | Difference with control ( 95%CI)\* | 7.45(-6.92, 21.83) | 33.25(8.54, 57.97) | 24.36(-9.11, 57.84) |
|  | *p*† | 0.296 | 0.010 | 0.167 |
| EOPE (N=17) | | 72.30(54.58, 109.66) | 370.00(132.37, 514.86) | 297.70(34.63, 454.05) |
|  | Difference with control ( 95%CI)\* | -2.62( -23.44, 18.19) | 191.25(52.33,330.16) | 196.49(63.67, 329.31) |
|  | *p*† | 0.791 | <0.001 | <0.001 |
| LOPE (N=49) | | 93.30( 53.80, 149.00) | 138.71(101.66, 207.00) | 30.63(0.00, 122.42) |
|  | Difference with control ( 95%CI)\* | 12.33(-5.03, 29.70) | 14.55( -11.16, 40.28) | -4.66(-37.17, 27.84) |
|  | *p*† | 0.162 | 0.245 | 0.766 |
| Mild PE (N=41) | | 103.45(53.80, 149.00) | 138.71(100.68, 207.08) | 34.63(-1.03, 130.69) |
|  | Difference with control ( 95%CI)\* | 13.71(-6.17, 33.60) | 15.00(-13.00, 43.02) | -3.70(-40.44, 33.04) |
|  | *p*† | 0.170 | 0.275 | 0.819 |
| Sever PE (N=25) | | 85.10(54.60, 109.66) | 168.24(128.14, 476.65) | 85.23(27.96, 340.35) |
|  | Difference with control ( 95%CI)\* | 0.15(-17.69, 17.99) | 78.93(26.88, 130.98) | 88.10(21.03, 155.17) |
|  | *p*† | 0.976 | 0.002 | 0.006 |

\* 95% CIs were estimated with the Hodges-Lehmann method.

† Based on Wilcoxon rank-sum test.

Abbreviation: TMAO, trimethylamine-N-oxide; PE, preeclampsia; EOPE, early onset preeclampsia; LOPE, late onset preeclampsia; T2, the second trimester; TD, the time of delivery; Q1, first quartile; Q3, third quartile; CI, confident interval