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METABOLOMIC ANALYSIS OF MOLECULAR SPECIES OF PHOSPHOLIPIDS FROM NORMOTENSIVE AND PREECLAMPTIC HUMAN PLACENTA ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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1. ABSTRACT

Electrospray ionization mass spectrometry (ESI/MS) is a highly sensitive, fast and powerful technique for the metabolite and metablomic analysis of mixtures of lipids in a biological extract. We have exploited this technique to identify and characterize various phospholipids present in the placenta of preeclamptic and normal pregnant women. Multiple major molecular species can be detected in each phospholipid class have arachidonic acid as major fatty acid constituent. There is no remarkable difference in the molecular composition in each of these phospholipids in both the extracts. However, there seems to be lower amounts of the plasmenyl phosphatidylethanolamine and greater amounts of free fatty acids in preeclamptic placenta.

2. INTRODUCTION

Biological membranes contain a diverse array of phospholipids (PHLs) as their major structural constituents (4). PHLs play an important role in membrane structure and function such as membrane fluidity, permeability, transport and signal transduction (22). The study of PHLs has clinical importance in that there exist several diseases with a component of an alteration in lipid metabolism (8, 11, 19). The typical structure of PHLs consists of a glycerol-3-phosphate backbone, and attached to the glycerol backbone are; a polar head group and two fatty acid chains esterified at Sn-1 and Sn-2 positions. In some instances, the substituent at Sn-1 position is alkyl ether or alkenyl ether (13). The PHLs containing alkenyl ether linkage at Sn-1 position are known as plasmalogens. This class of PHL molecule are antioxidant molecules that can protect cells from oxidative stress (2). The PHLs are classified according to the specific hydrophilic head group that includes water, choline, ethanolamine, serine and inositol. Since the fatty acid chains can vary in length and degree of unsaturation, each PHL class contains numerous molecular species. These species differ greatly in their chemical and biological properties and occur in characteristic proportions in different organs and developmental stages and wide variety of acute and chronic pathologies (12, 27). Because of the great diversity of the PHLs and the fatty acid chain length, there is a wide variety of combinations that can come together to form membrane phospholipids. It is because of this diversity of PHL structures that a metabolomic analytic approach is needed (6).

There have been many studies of PHL molecular species in membranes. Characterization by traditional techniques (TLC, HPLC, derivatization followed by GC) (20, 26) is difficult due to large number of individual molecular species that can occur in a tissue and produce confounding signals. This can result in underestimation of labile species lost during multiple processing steps. This may also result in artifactual generation of oxidized species ex vivo in PHLs, containing polyunsaturated fatty acids

(23). Thus, it is desirable to characterize intact PHL molecules from the tissue directly to prevent alteration or degradation of the sample.

ESI/MS has been shown to be very sensitive and powerful technique (3, 16) for rapid characterization of PHL classes, to identify unique individual molecular species within each class and thereby to assess alterations in the PHL content in any biological extract (15). PHLs differentially acquire either positive or negative charge under the ESI source high voltage, so both, positive and negative ion ESI/MS are used for the structural and metabolomic characterization of molecular species of intact PHLs (29, 31). In this study, we have utilized ESI/MS to identify and characterize Phosphatidylethanolamine (PE), Phosphatidylinositol (PI), Phosphatidylserine (PS), Phosphatidylcholine (PC), Sphingomyelin (SM), Phosphatidic Acid (PA), the major class of PHLs found in placenta (1).

3. METHODS

3.1. Experimental Procedures

Patients. Placentas from five normal pregnant women (NTP) and three preeclamptic patients (PEP) were received from local hospitals. Patient confidentiality was maintained. Institutional Review Board (IRB) approval was obtained and protocols for maintaining confidentiality were observed. The diagnosis of PEP was made by blood pressure of 140/90 mm Hg with proteinuria (urinary protein >300 mg/24-hour urine collection).

3.1.2. Materials

All Placentas were collected immediately after delivery and snap frozen using liquid nitrogen. The tissue was stored in a freezer at –70°C until analysis. Standard PHLs were obtained from Avanti Polar Lipids. All organic solvents used were of analytical grade and obtained from Fischer Scientific. HPTLC and preparatory (0.5 and 1.0 mm thickness) TLC plates, coated with silica from Whatman (New Jersey, USA), were used.

3.2. Preparation of placental extract

The placental extracts were prepared using a modification of the Folch et al method (9). Approximately 1g of placental tissue was homogenized with 20 ml of ice-cold Chloroform:Methanol (C:M) 2:1 solution containing 0.005% BHT, using a blade homogenizer at full speed for 60 sec. The air space in the centrifuge tube was purged with nitrogen and the solution was left over ice for 1 hour to effect maximum extraction of lipids from ground tissue. The tube was vortexed occasionally during this period of time. After adding 4 ml of 0.9% aqueous NaCl, the tube was shaken vigorously for 2 min and centrifuged at 1000g for 10 min. The lower organic layer containing lipids was collected and dried under nitrogen and used for analysis by ESI/MS and TLC/ESI/MS.

3.3. TLC separation and isolation of major PHL classes from placental extracts

PEP and NTP extracts prepared with ~1 gm of solid tissue and dissolved in ~1 ml of solvent, C:M (2:1) are subjected to high performance 1D and 2D-TLC in a purpose built dry box. The dry box is degassed to remove O2 and the gas replaced with nitrogen. Both samples are run simultaneously in this system using identical conditions. The solvent system used for TLC separation in the first dimension consists of C:M:Water (C:M:W) 65:25:4.5 and C:M:7MNH4OH 65:35:5 in the second dimension. To confirm the consistency of the extraction procedure, both 1D and 2D-TLC plates are stained with the following: iodine, molybdate and ninhydrin. Most lipids and PHLs are detected with iodine vapor (30). Molybdate is used to detect any phosphorus-containing compound only (e.g. all PHLs)(7). Ninhydrin is used to detect only PHLs, containing free amino group (e.g. PE, PS and their lyso derivatives) (30). Major PHLs observed on 1D-TLC plates are tentavely identified by comparing Rf values of unknown bands with known PHL standards. PHL classes are subsequently separated by preparative 2D-TLC using 1mm plate. All major spots observed on the 2D-TLC chromatogram are identified by ESI/MS. Each spot is scraped and extracted in C:M:W (5:5:1) three times. Supernatants are combined and dried under nitrogen. Lipids are extracted and collected by adding equal volume of C and W. The chloroform phase is collected, and dried under nitrogen

3.3.1. Mass Spectrometry

The dried PEP and NTP extracts, were diluted in C:M (1:2) with or without NH4OH. The sample was through a syringe pump (Howard Apparatus) at the rate of 8µl/min into the ESI source and the data acquired for 1 minute. Approximately ~54 scans are averaged during this period of time. Figure. 1 illustrates the strategy used for global analysis of PHLs in the extract by ESI/MS without the need for prior chromatographic separation. ESI/MS analysis was performed on quadrupole time of flight mass spectrometer (QTOF/MS), Waters Corporation. Data were acquired using MassLynx NT software, Waters Corporation.

3.4. Results and discussion

Preeclampsia is a relatively common complication of pregnancy thought to arise from the production of abnormal PHLs (25) in the placenta of preeclamptic women. There are reports describing the placental PHL contents and molecular species, although multistep processes for extraction and analysis were employed (1, 24). These techniques were employed before the advent of the relatively new metabolomic methods (6). The objective of this study is to use metabolomic analysis to determine the PHL profile in the placental extracts of normal pregnant women (NTP) and preeclamptic women (PEP) and identify different molecular species in each PHL class in both the extracts (31). We have identified and characterized major PHL species analyzing directly placental extracts by ESI/MS and also, assessed the effect of chromatographic separation of the placental extracts using TLC followed by ESI/MS of each separated spot.

4.ESI/MS OF TOTAL NTP AND PEP LIPID EXTRACTS

4.1. Positive ion ESI/MS

Figure 2 (a-c) illustrates the total positive ion

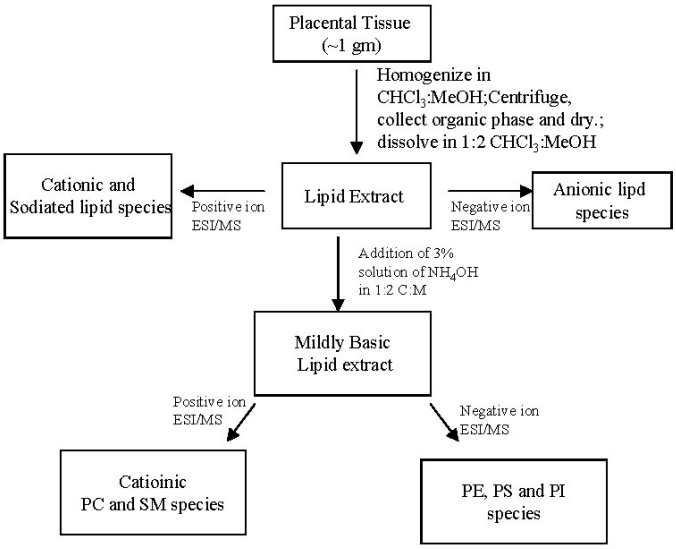

Figure 1. Schematic diagram of the experimental strategy used for metabolomic analysis of placental PHLs directly from placental extracts without the need for prior chromatographic separation.

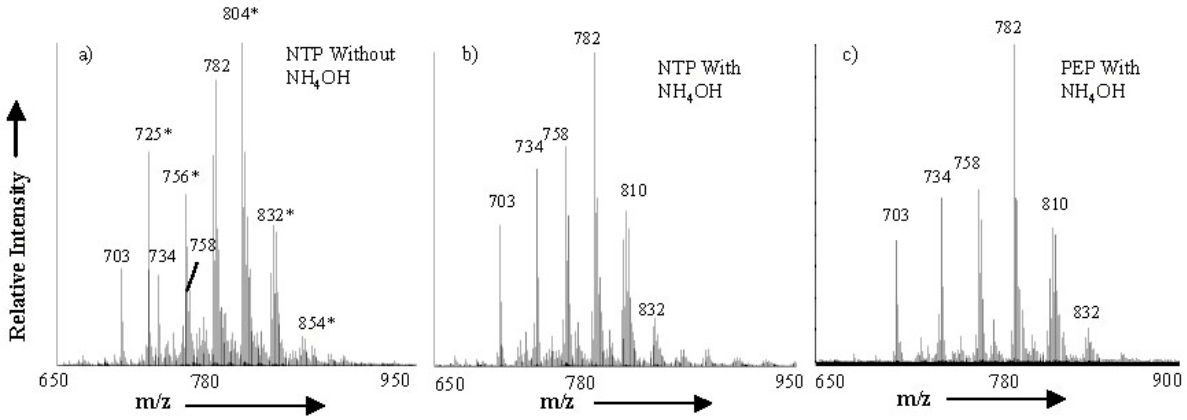

Figure 2. Positive ion ESI/MS of placental lipid extract; (a) NTP extract dissolved in 1:2 C:M; Sodiated species are indicated by asterisks. (b) NTP extract dissolved in 1:2 C:M containing 3% NH_4OH; (c) PEP extract dissolved in 1:2 C:M containing 3% NH_4OH ; Note there is essentially complete elimination of all sodiated PC and SM (725) peaks with the inclusion of NH_4OH in the infusion solvent in b and c.

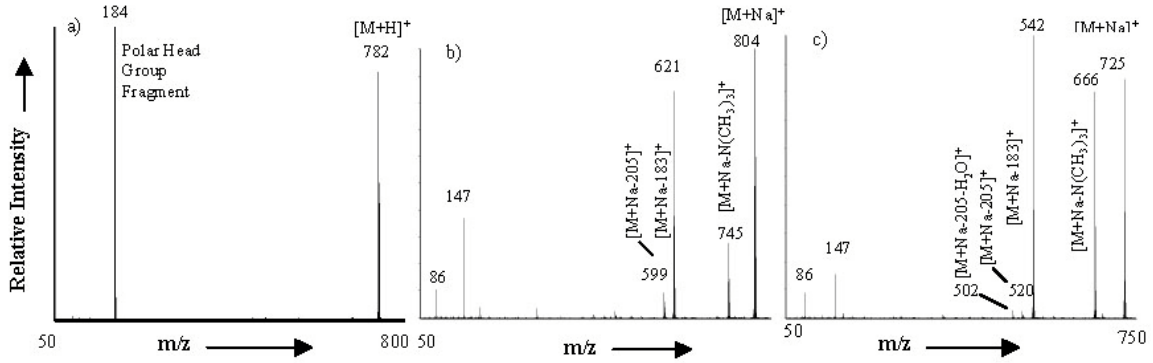

Figure 3. Product ion spectra of protonated (782), sodiated PC (804) and SM (725) species. (a) Protonated PC (782) fragments to give abundant phosphocholine ion at 184. (b) Sodiated PC species (804) is characterized by the presence of ions corresponding to the loss of trimethylamine (N-(CH3)3 (59) , choline phosphate (621) and sodiated choline phosphate (599). (c) Sodiated SM species shows similar corresponding product ions at 666, 542 and 520 and an additional ion corresponding to the loss of H_2O from sphingosine observed at 502.

current profile from ESI/MS analysis of PHLs from PEP and NTP extracts in the absence and presence of NH4OH. The ESI mass spectra show almost exclusively molecular ion species of polar lipids. Most of the ions observed in Figure 2 have even nominal m/z values, indicating that they contain an odd number of nitrogen atoms. The only exception are the ions at m/z 725 in Figure 2a and m/z 703 values in Figure 2b and c that represent PHL species containing even number of nitrogen atoms. Positive ion spectrum (Figure 2a) is complicated by the presence of two different ions [M+H]+ and [M+Na]+ of PC and SM. This phenomenon greatly complicates the analysis of PHLs in any biological extract. The addition of NH4OH in the infusion solvent (21), eliminates the sodium adducts of PC and SM ions and increases the abundances of these ions as shown in Figure 2b and c, thus greatly simplifying the interpretation of spectra.

Collision activated decomposition (CAD) of a selected peak in positive ion mode provides information about head group moiety, thereby identifying a particular PHL class. CAD of all peaks in Figure 2 b and c gave single intense fragment ion at m/z value 184, corresponding to the polar head group of PC and SM. Ion at m/z 703 is easily identified as SM due the presence of extra nitrogen giving it odd nominal mass. Positive ion spectra of sodiated PC and SM yielded dramatically different CAD patterns from the protonated species. Product ion spectrum of all sodiated PC and SM ions displayed four abundant ions. Figures 3a, b and c show ESI/MS/MS spectra of m/z 782 (protonated PC) and m/z 804 (sodiated PC) and m/z 725 (sodiated SM) species, respectively. Each of the product ions of sodiated PC (Figure 2a) corresponds to the loss of trimethylamine ([M+Na-59] +), the production of diglyceride cations ([M+Na-183] + and [M+Na-205]+) and the generation of a five-member sodiated cyclophosphane (NaOOPOOHCH2CH2;m/z 147) . In contrast, the CAD of the protonated species (Figure 3a) resulted in only a single phosphocholine cation, m/z 184. The same product ion pattern was observed in CAD of other protonated (m/z 810, 734, 758, 832) and sodiated (m/z 832 and 756, 780 and 854) PC species as well as in CAD of protonated (m/z 703) and sodiated (m/z 725) SM species. A distinguishing product ion in ESI/MS of sodiated SM species as compared to those of sodiated PC is the presence of an ([M+Na-205 – H2 O] +) ion due to the loss of H2 O from the sphingosine (14).

Characterization of molecular species of observed PC and SM requires fatty acid analysis, done in negative ion ESI/MS/MS as discussed below.

4.2. Negative ion ESI/MS

ESI/MS in negative ion mode provides opportunity to study mixture of fatty acids and complex lipids. The negative ion spectra of placental extract in Figure 4 shows intense ions of the [M-H]- of PE, PI, PS and PA. Negative ion spectrum (Figure 4a) is complicated by the presence of intense ions due to chloride adducts of PC and SM . Chloride ions present in biological lipid extracts give rise to negatively charged adduct ions of PC and SM which are observed as [M+Cl]- ions. The addition of NH4OH in the infusion solvent (Figure 4b and c) not only eliminates these adduct ions but also increases the abundances of PE ions as shown in Figure 4 b and c, thus greatly simplifying the interpretation of spectra.

On the basis of the m/z value and fatty acid composition as observed in negative ion ESI/MS/MS spectra, the major ions in Figure 2, and 4 can be correctly assigned to individual PHL molecular species of PC, PE, PI, PS, PA and SM. These assignments are summarized in Table 1. Characterization of all major PHL species in Table1, shows that all PHL classes predominantly have arachidonyl substituent.

4.3. ESI/MS/MS of Choline and Sphingomyline phospholipids

In Figure 5 we see the product ion spectrum of the peaks at m/z 816. The most abundant fragment ion is found at m/z 766 which is formed by neutral loss of CH3Cl (50 dalton (D)) and represents demethylated PC detected as [M-H]- ions. Other fragments at m/z 255, 279 and 303 represent fatty acid anions of 16:0, 18:2 and 20:4 respectively. Fragment ion at m/z168 represents the demethylated polar head group of PC species. This indicates PC (36:4), and the intensity distribution of these ions show that PC (36:4) is present as a mixture of PC (16:0/20:4) as the main component accompanied by a minor component of PC-(18:2/18:2). The species seen at m/z 480 indicates neutral loss of arachidonate as a substituted ketene. Similarly, the fatty acid composition of other PC molecular species in Figure 4a are determined and summarized in Table 1. The product ion spectrum of m/z 737 (Figure 4a) showed most abundant fragment ion at m/z 687 which is formed by neutral loss of CH3Cl (50D) and represents demethylated SM detected as [M-H]- ions. The only other fragment is observed at m/z 255 is due to fatty acid anions of 16:0 (data not shown).

4.4. ESI/MS/MS of Inositol phospholipids

Figure 5c represents the product ion spectrum of most abundant ion (m/z 885) of PI molecular species. It is characterized by the presence of polar head group fragment at m/z 241which represents the dehydration product of inositol phosphate. Fatty acid anions of m/z 283 and 303, ions reflecting neutral loss of arachidonate as free fatty acid (m/z 581) and an ion (m/z 419) reflecting neutral loss of inositol (162 D) from m/z 581, are also present. Similar product ion spectra are obtained from m/z 857, 833 and 835.

4.5. ESI/MS/MS of Serine phospholipids

The ions at 810, 788, 786, 834 and 836 (Figure 4b and c), corresponds to PS species. The product ion spectrum of m/z 810 is shown in Figure 6a. The spectrum shows m/z 283 and 303 anions, a phosphatidate anion (m/z 701, loss of 87 D) reflecting loss of dehydration product of serine from the [M-H]- ion. Other ions reflecting neutral loss of arachidonate as free fatty acid (m/z 419) or as a substituted ketene (m/z 437) from the phosphatidate ion are also present. Analogous product ions (neutral loss of 87D) are observed in product ion spectra of peaks of PS at m/z 788, 834 and 836.

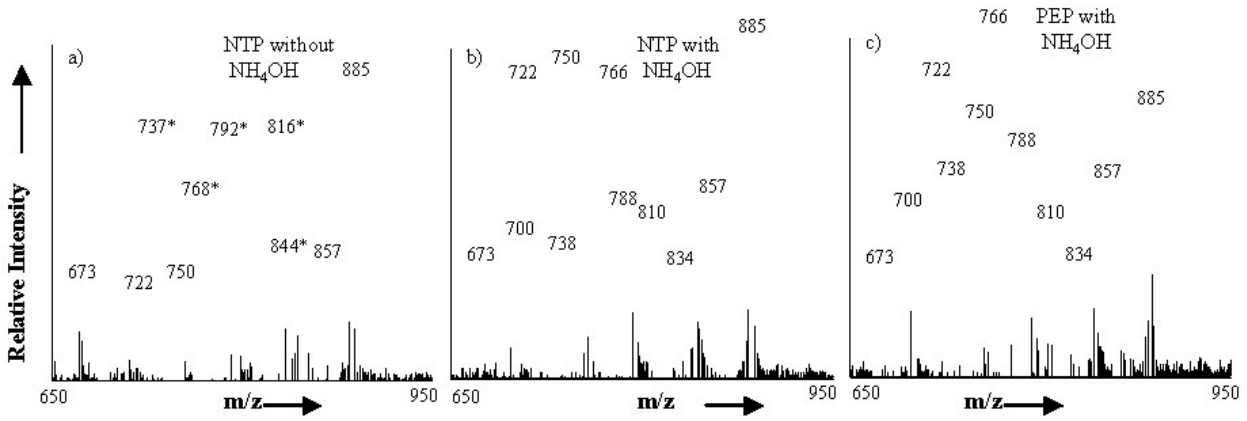

Figure 4. Negative ion ESI/MS of placental lipid extracts. (a) NTP extract dissolved in 1:2 C:M. Peaks marked with asterisks represent chlorine adducts of PC and SM peaks. (b) NTP extract dissolved in 1:2 C:M containing 3% NH_4OH. (c) PEP extract dissolved in 1:2 C:M containing 3% NH_4OH. Addition of NH_4OH in the infusion solvent in the panels b and c, brings up the intensity of all PE peaks (Table 1) and eliminates all major PC and SM peaks marked with asterisks that are observed in panel a.

Table 1. PHLs species identified by ESI/MS

PC					
$[M+H]^+$	**$[M+Na]^+$**	**$[M+Cl]^-$**	**S_{n-1}/Sn_{-2}**	**Minor $[M+H]^+$**	**S_{n-1}/Sn_{-2}**
782	804	816	16:0/20:4	834	18:1/22:5
784	806	818	16:1/20:4	832	18:1/22:6;
758	780	792	16:0/18:2	830	18:2/22:6;
760	782	794	16:0/18:1	746	16:0e/18:1
734	756	768	16:0/16:0	720	16:0e/16:0
808	830	842	18:1/20:4	854	20:4/22:6
810	832	844	18:0/20:4	706	14:0/16:0
806	828	840	18:2/20:4	768	16:0e/20:4
PE					
$[M+H]^+$		**$[M-H]^-$**	**S_{n-1}/Sn_{-2}**	**Minor $[M-H]^-$**	
768		766.5	18:0/20:4	742	18:1/18:1
752		750.5	18:0p/20:4	744	18:0/18:1
724		722.5	16:0p/20:4	788	18:1/22:6
750		748.5	18:1p/20:4	790	18:0/22:6
740		738.5	16:0/20:4;	774	18:0p/22:6
				700	16:0p/18:1
PI					
		885	18:0/20:4	833	16:0/18:2
		857	16:0/20:4	835	16:0/18:1
PS					
812		810	18:0/20:4	834	18:0/22:6
790		788	18:0/18:1	836	18:0/22:5
788		786	18:0/18:2		
PA					
		673	16:0/18:1		
		723	18:0/20:4		
Phosphatidyl Glycerol (PG)					
		747	16:0/18:1		
Phosphatidylethanol (PEth)					
		673	16:0/16:1		
		701	16:0/18:1		

4.6. ESI/MS/MS of Ethanolamine phospholipids

The major contributors to the peaks at m/z 700, 716, 722, 738, 742, 748, 750, 766, 774 and 790 (Figure 5b and c) are PE species and two series of compounds are represented, among them. The first consists of diacyl-PE species and the second of plasmalogen species, which dominates the spectra. Figure 6b represents the product ion spectrum of peak at m/z 788. The most abundant product ions are the fatty acid anions at m/z 281, 283 and 327. Also, there is a peak at m/z 701 corresponding to loss of 87

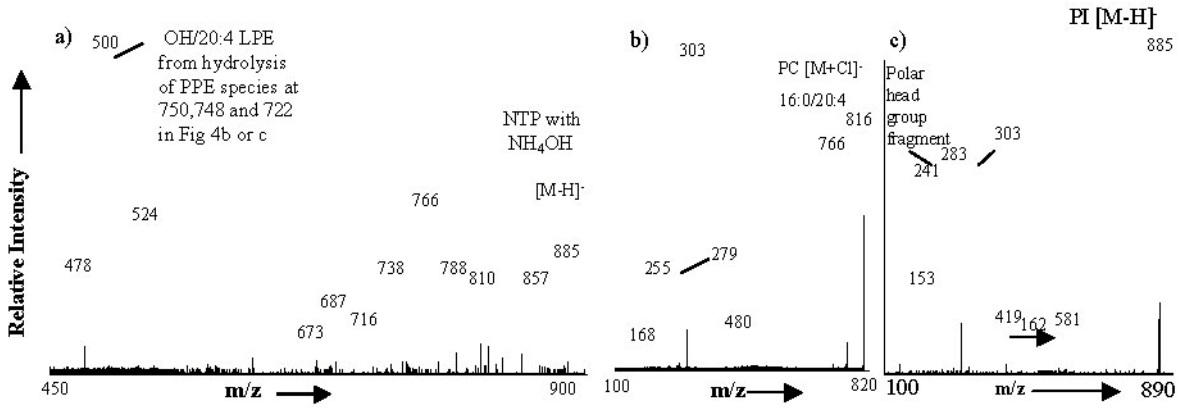

Figure 5. (a) Negative ion ESI/MS from NTP extract. The homogenized tissue was left at room temperature for an hour for maximum extraction of lipids, which resulted in hydrolysis of PPE species (750, 748, 722, 700, 774), which are absent here and present in Figure 4b and c above. (b) Product ion spectrum of abundant chloride adduct of PC species in Figure 4a above. The formation of ion at 766 is due to the loss of CH_3Cl from chloride adduct $[M+Cl]^-$ of the most abundant ion at 782 in the positive ion spectrum of extracts (Figure 2). (c) Product ion spectrum of PI (18:0/20:4) at 885 in Figure 4 is the diagnostic peak which corresponds to the dehydration product of the polar head group fragment at 241.

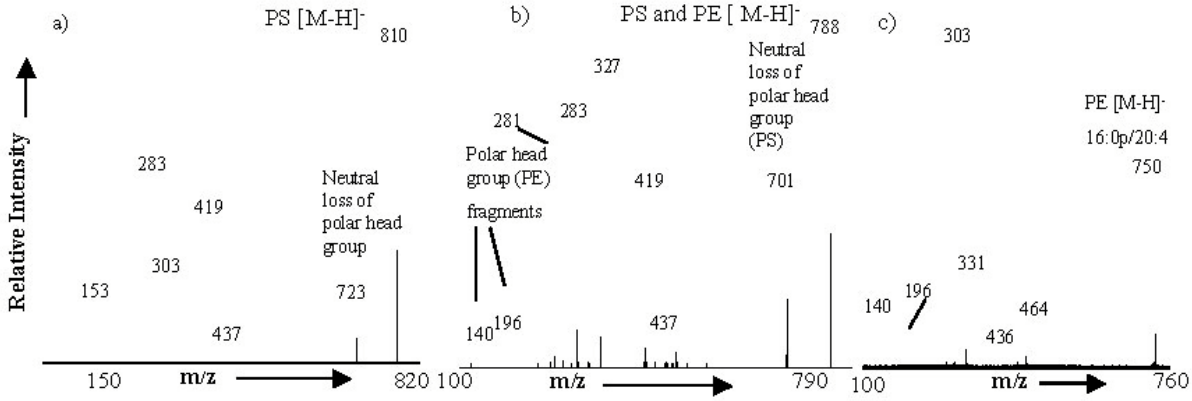

Figure 6. Product ion spectra of ESI/MS of PHL ions in Figure 4. (a) Product ion spectrum of deprotonated ion of PS at 810 (18:0/20:4). (b) Product ion spectrum of the deprotonated ions of isobaric species 788 of PS (18:0/18:1) and PE (18:1/22:6). Neutral loss of 87 reflects the loss of dehydration product of serine from $[M-H]^-$ as in panel a. Diagnostic PE peaks correspond to the polar head group fragment at 140 and dehydration products of glycerol phosphoethanolamine at 196. (c) Product ion spectrum of PPE at 750 shows one major carboxylate ion at 303. This species is identified as 18:0p/20:4 PPE. The small carboxylate ion at 331 shows the presence of a minor isobaric PPE species (16:0p/22:4).

D (characteristic of PS). There are two diagnostic peaks to identify PE ions at m/z 140 (polar head group) and at m/z 196 (glycero phosphoethanolamine-H2O). Thus, the signal at m/z 788 is due to the isobaric species, containing the same molecular weight (but different head groups thus forming different phospholipids), of PS (18:0/18:1) and PE (18:1/22:6). Product ion spectra of other PE peaks (m/z 716,738,766, 742 and 790) showed fragments at m/z140 and196 and fragments corresponding to two fatty acid anions in each, identifying them as diacyl PE species. However, the product ion spectrum of signal at m/z 750 showed prominent ion at m/z 303 and ions reflecting neutral loss of arachidonate as free fatty acid (m/z 446) or as a substituted ketene (m/z 464). It is consistent with the PPE (plasmenyl phosphatidylethanolamine) species (18:0p/20:4). There is also a small fatty acid anion at m/z 331 reflecting the presence of a minor isobaric PPE species (16:0p/22:4). Similar product ions of the peaks at m/z 748, 722, 700 and 774 are consistent with PPE species as given in Table 1. There is a remarkable difference in the intensity of PPE peaks between the PEP and NTP. This deficiency of PPE in case of preeclamptic sample may be responsible for oxidative stress and pathogenesis of preeclampsia.

Assignment of the indicated species as plasmalogens is confirmed by negative ion ESI/MS of NTP extract, prepared by leaving the homogenized tissue for an hour at room temperature rather than over ice. All peaks corresponding to PPE species are absent and corresponding Sn-1lyso derivatives (LPE) peaks are present (Figure 5a). Plasmalogens are more susceptible to hydrolysis and easily destroyed by mild acid treatment (28). Leaving the tissue homogenate at room temperature resulted in the hydrolysis of PPE species, producing LPE species at m/z 478, 500 and

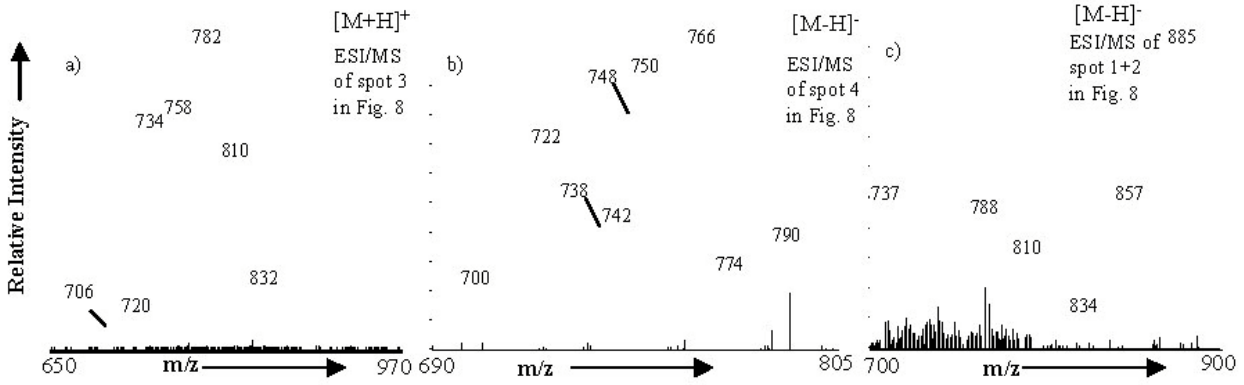

Figure 7. ESI/MS of extracts of TLC derived spots (similar to that obtained from traces seen in Figure 8) from the separated PHLs on 2D-TLC plate. (a) Positive ion ESI/MS of spot 3 (dissolved in C:M; 1:2 containing 3% NH_4OH) showing all PC species as observed in Figure 2b and c without prior chromatographic separation. (b) Negative ion ESI/MS of spot 4 (dissolved in C:M; 1:2 containing 3% NH_4OH) showing PE species as observed in Figures 4b and c without prior chromatographic separation. (c) Negative ion ESI/MS of spot1+2 (dissolved in C:M; 1:1) showing species corresponding to PI, PS and SM+Cl (737) as observed in Figures 4a, b and c without prior chromatographic separation.

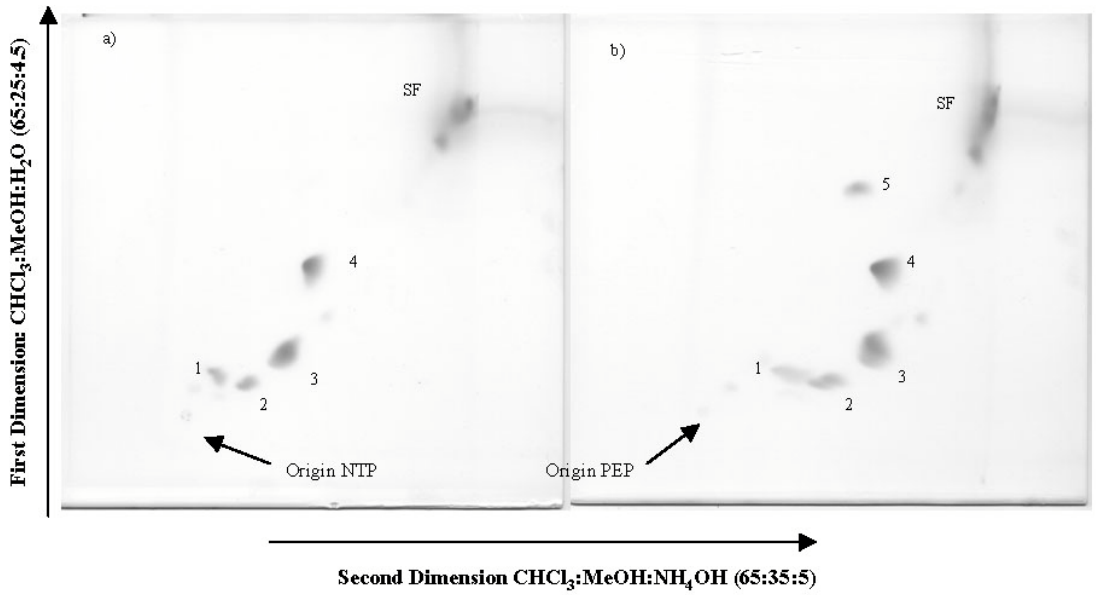

Figure 8. 2D-HPTLC chromatogram of normotensive (a) and Preeclamptic (b) extracts as described in the text. The extracts were prepared in identical manner containing the same amount of dry solid and dissolved in same amount of solvent (C:M 2:1) to allow direct comparison, prior to application on the TLC plate. Major spots are identified as 1: PI and PS; 2:SM; 3:PC; 4:PE; 5: Free fatty acids; SF: solvent front. There is no demonstrable difference between the PHLs in the two extracts. However, free fatty acids (spot 5) are absent in NTP extract.

524. Product ion spectra of peaks at m/z 500, 478 and 524 confirmed them as OH/20:4, OH/18:1 and OH/22:6 LPE species respectively.

4.7. 2D-TLC separation and identification by ESI/MS

Figure 7 (a-c) shows the ESI/MS of separated spot extracted from TLC of NTP extract. Similar results are found from 2D-TLC of PEP extract. Positive ion ESI/MS (Figure 7a) of extract of spot 3 demonstrates multiple predominant ion peaks corresponding to PC molecular species. Spot 4 and (1+2) were identified by negative ion ESI/MS and are illustrated in Figures 7 b and c respectively. ESI/MS of spot 4 shows peaks corresponding to PE species and spot (1+2) demonstrates predominantly multiple peaks corresponding to molecular species of PI, PS and SM species. The molecular composition of all major PHL species PC, PI, PS, SM and PE obtained by TLC/ESI/MS (Figure 7a-c) is found to be identical to the PHL species obtained by direct ESI/MS/MS analysis of either extract as given in Table1. TLC was performed in a dry box under nitrogen, to protect PHLs from auto-oxidation. Figure 7 (a-c) shows that there is no selective loss or alteration of any PHL molecular species composition that might occur during chromatography, as observed by Delong et al (5). Figure 8 illustrates the 2D-HPTLC chromatogram of NTP and PEP extracts showing several coincident spots. There does not seem to be any

remarkable difference between spots 1, 2, 3 and 4. The only difference is found in the intensity of spot 5, which is almost absent in NTP. This spot is identified as mainly containing arachidonic acid (AA), by ESI/MS. The increase in free fatty acids in PEP extract, may result from an increase in the activity of placental phospholipase A2, which is increased in Preeclampsia (10, 18). Free AA is a precursor molecule for potent biological mediators and is easily oxidized to form potent vasoconstrictors such as thromboxanes and isoprostanes, whose levels are increased in preeclampsia (32, 33).

Other minor PHLs identified by negative ion ESI/MS are PA, phosphatidyl glycerol (PG) and phosphatidyl ethanol (PEth), based on their m/z values, as given in Table1. ESI/MS/MS spectra of PA and PG in the negative ion mode produce abundant fatty acid ions and PEth also produces head group fragment at m/z125.

There are complications of interpretation from isobaric species, in the negative ion mode even with the addition of NH4OH in the infusion solvent, but they are minor species in the current extract. Product ion analysis in this situation gives three or four fatty acid anions as shown by CAD of m/z 788 (Figure 6b). But by looking at the polar head group fragments and fragments due to neutral loss of polar head group, it is possible to predict the fatty acid composition of overlapping PHL species based on the m/z values. This information becomes important metabolomic data for understanding the lipid metabolite fingerprint observed from placenta and placental extracts (31). Online LC/MS (17) or Precursor ion scans (3), in this is particularly useful for metabolomic analysis of placental extracts.

5. CONCLUSION

Molecular composition of all major PHL species PC, PI, PS, SM and PE were found to be identical in PEP and NTP extracts. However, there seems to be a PPE deficiency and increase in levels of free fatty acids, in PEP as observed by ESI/MS analysis. Metabolomic analysis of all major molecular species of all PHLs identified showed the presence of esterified AAs. Addition of NH4OH in the infusion solvent, enables PC and SM species to be identified by positive ion ESI/MS and PE, PS, PI and PA by negative ion ESI/MS. Thus, metabolomics using ESI/MS provides powerful, rapid and highly sensitive method for complete analysis of PHL classes, subclasses and individual molecular species in order to characterize the lipid content in biological tissue, without the need of prior chromatographic separation or derivatization.

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