Metabolic profiling of milk in preeclampsia patients & healthy controls: An in-vitro NMR study

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Introduction: Preeclampsia (PE) is a pregnancy specific syndrome and is associated with vascular pathology, which may affect lactogenesis[1]. This may lead to altered milk components that influence the infant's growth and development. It is known that PE is associated with foetal growth restriction[2] and cognitive disorders[3] in offsprings in later life. Thus, the objectives of the present study are: (a) to analyze the metabolome of milk in mothers with PE using in vitro-NMR spectroscopy, and (b) to identify the metabolites that can differentiate the PE group from control women.

Patients and Methods: Twenty six women with PE (n=26) were recruited for this study. Thirty-one pregnant women (n=31) with no medical and obstetrical complications served as controls. Written informed consent was taken from each subject and the Institute Ethics Committee approved the study. Milk samples were collected on day 3 and 6 months of lactation. Samples (2 ml) were taken at the end of breast-fed and collected into cryo vials and stored at −80°C until further analysis. For NMR spectroscopy, samples were thawed and shaken thoroughly to homogenize. Fifty microlitres of milk was mixed with 520 µL of D_2O. Formate (0.5 mM) was added to the sample that served as a concentration reference for ^{1}H NMR studies. One dimensional (1D) and two dimensional (2D) total correlation spectroscopy (TOCSY) NMR experiments were carried out at 700 MHz (Agilent, U.S.A.). The typical parameters for 1D experiment were: spectral width of 9124.1 Hz; data points 32 K; number of scans 16 and relaxation delay of 70 seconds. The following parameters were used for 2D NMR experiments: data points 2 K in F2 dimension; spectral width 9124.1 Hz and relaxation delay of 2 seconds. Mixing time of 80 ms was used for TOCSY experiments. The1D NMR spectra were subdivided into 0.02 ppm integral regions using the Chenomx NMR Suite 7.5 software (Chenomx Inc. Edmonton, Canada) to reduce the spectrum into 200 variables in the region of 0.5-4.5 ppm. Principle component analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) was also carried out on the binned data of the metabolites to discriminate between samples of diseased and healthy subjects using Unscrambler 10.2 (CAMO, Oslo, Norway).

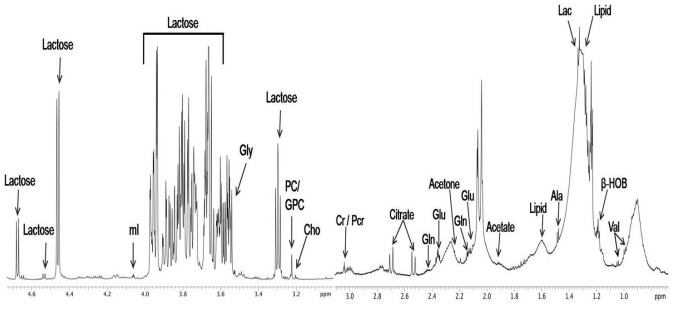

Figure1: Representative ^{1}H NMR spectra of milk of mother with PE.

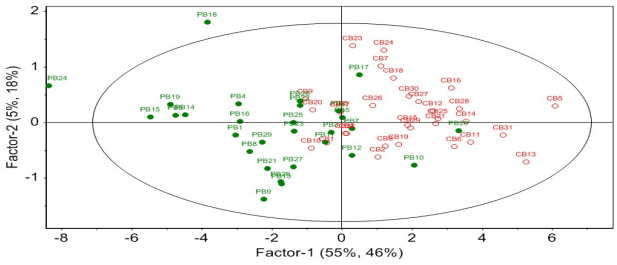

Figure2. PLS-DA plot differentiates women with PE (●) and controls (o) at day 3 of lactation

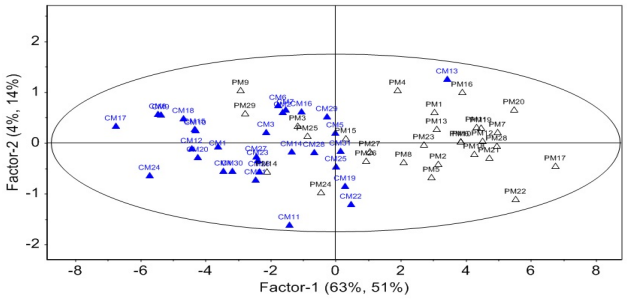

Figure3. PLS-DA plot differentiates women with PE (▲) and controls (Δ) at 6 month of lactation

Results: Figure 1 shows the representative aliphatic region of the ^{1}H NMR spectra of milk of a patient with PE. In all 17 metabolites were assigned unambiguously assigned using 1D and 2D NMR. The PLS-DA scores plot (Fig.2) exhibited that preeclampsia group is clearly separated from control group at day 3 of lactation and the model showed the cumulative explained variance of (R^2) of 0.94 and cross validated predictive fraction (Q^2) of 0.61. The loading plot indicated that the variables corresponding to glycine (Gly) and glycerophosphocholine (GPC) were responsible for the seperation between the two groups. PLS-DA was also performed using the milk spectra of PE and control group at 6 months of lactation (Fig.3) which showed R^2 value of 0.93 and Q^2 of 0.61. The resulting score plot demonstrated the clear separation of PE group from control group at 6 month of lactation and the loading plot indicated that the variables corresponding to GPC was responsible for the separation of the two groups.

Discussion: To the best of our knowledge, this is the first study that presented comprehensive biochemical characterization of milk across the lactation period in mothers with PE and control women. Our data revealed there is an alteration in levels of GPC and Gly in milk of mothers with PE as compared to controls. Gly acts as an inhibitory neurotrasmitter and required for the brain development in infants. The lower level of Gly at day 3 of lactation suggests that there is inadequate supply of Gly to infants born to PE mothers and may affect infant's growth and particularly the brain development. Further, our results showed lower GPC level in the milk of mothers with PE indicating altered choline metabolism. Altered choline level affects the production of phosphatidylcholine which in turn influences the delivery of docosahexaenoic acid to the foetus where it is important for fetal brain development[4]. This affects the brain development of the infants born to mothers with PE.

Conclusions: The milk components such as GPC and Gly levels were altered in mothers with preeclampsia. These altered milk components in mothers with PE may be associated with neurodevelopment of the infant and may affect the cognitive functions in babies born to mother with PE.

References: (1) Erbaˇgci AB et al. *Clin Biochem*, 2005; 38: 712–716. (2) Srinivas SK et al. *J Perinatol*, 2009; 29: 680–684. (3) Many A et al. *Hypertens Pregnancy*, 2003; 22: 25–29. (4) Zeisel SH et al. *Annu Rev Nutr*, 2006; 26:229–250.