Research Article OPEN ACCESS | Freely available online

Examination of the 1st trimester maternal plasma proteome by SELDI in pregnancies with Pre-eclampsia

Varaprasad Kolla[1*], Narasimhan Kothandaraman[2], Irene Hoesli[3], Mahesh Choolani[4], Sinuhe Hahn[3] and Olav Lapaire[3]

[1]School of Life & Allied Sciences, ITM University, Naya Raipur, India. [2]Centre for Excellence in Genomic Medicine Research, King Fahd Medical Research Centre, King Abdul Aziz University, P.O. Box 80216, Jeddah 21589, Kingdom of Saudi Arabia. [3]University Women's Hospital, University of Basel, Hebelstrasse 20, Switzerland. [4]Biomarker Discovery Laboratory, Department of Obstetrics and Gynaecology, National University of Singapore, Singapore.

Abstract: Currently no reliable 1st trimester screening method exists to detect for pregnancies, which will develop preeclampsia. This deficit makes it difficult to develop appropriate intervention strategies. As the underlying placental aetiology leading to the development of preeclampsia is thought to occur early during gestation, we hypothesized that such changes may already be evident in the maternal plasma proteome. Hence, such unique proteomic fingerprints could be used to distinguish between pregnancies with healthy deliveries from those, which developed preeclampsia. For this purpose we investigated the use of Surface-Enhanced Laser Desorption/Ionization Time-of-Flight mass spectrometry (SELDI-TOF-MS) SELDI. Unique spectral profiles were generated using SELDI-TOF-MS. A total of 8 peaks corresponding to peptides and proteins in the range between 3.2 kDa and 22.2 kDa were identified from a set of 26,707 representing the whole spectral analysis (0 to 60 kDa), were identified which could discriminate pregnancies with normal deliveries to those which developed preeclampsia. Maternal plasma proteome fingerprinting by SELDI-TOF MS may lead to the development of a set of markers which can be used in discern pregnancies at risk for pre-eclampsia.

Keywords: SELDI-TOF MS, Pre-eclampsia, 1st trimester, placenta, pregnancy, plasma proteomics.

Citation: Varaprasad Kolla et.al. (2018) Examination of the 1st trimester maternal plasma proteome by SELDI in pregnancies with Pre-eclampsia. Journal of PeerScientist 1(1): e1000003.

Received June 08, 2017; **Accepted** February 27, 2018; **Published** March 15, 2018.

Funding: This research work is supported by FP6 funded *PREGENESYS* project of European commission grant # 037244.

Competing Interests: Authors whose names are listed in this manuscript are affiliated or involved in an organization or entity as disclosed above with a financial or non-financial interest in the subject matter or materials discussed in this manuscript.

* **E-mail:** varaprasadk@itmuniversity.org, naidu.prasad@gmail.com **Phone**: +91 9850022124

I. INTRODUCTION

In the last two decades several biochemical and biophysical traits were identified which led to a considerable improvement in the screening of preeclampsia (PE). But there is no reliable screening biomarker for PE. So there is a need in improve both the sensitivity and specificity of current screening strategies. As considerable efforts has being invested in the discovery and development of further markers. Platforms based on proteomics [1] coupled with mass spectrometry (MS) techniques [2] is gaining considerable acceptance following the identification of different markers associated with several types of disease conditions such as ectopic pregnancies [3], detection chromosomal abnormalities [4-5], ovarian cancer [6-7], diabetic nephropathy [8] and inflammatory bowel disease [9]. Till date only few protein markers are available for the early screening of pregnancy for PE. The current practice of using single protein biomarkers will most likely give way to the use of multiplexed biomarkers, as they promise better sensitivity and specificity [10]. High-throughput platforms using MS permit profiling large numbers of samples in relatively shorter time mostly within the same day of admission. Using proteomics platform to mine deep into the plasma proteome has resulted in the identification of several target candidates for Down's syndrome [11-12]. Moreover proteomic signatures can reduce problems with individual variability in peak detection [13]. We hypothesized that by using a combination of unique serum proteomic features (hydrophobic proteins) in protein profiles and differentially expressed proteins (hydrophilic proteins) could be employed to distinguish pregnancies at risk of developing preeclampsia from control healthy conditions.

II. Results and Discussion

Unique spectral profiles were generated using SELDI-TOF-MS method (Figure1). A total of 8 spectral peaks corresponding to peptides and proteins in the range between 3.2 kDa and 22.2 kDa were identified from a set of 26,707 features representing the whole spectra (0 to 60 kDa) (Table 2). Eight features were identified which could discriminate all control pregnancies from the PE – before onset of PE. We found Galectin1 (P09382) and Plasminogen (P00750) (Table 3) are the protein identified in our pervious study using quantitative proteomics and protein profile found in our current study. Using SELDI platforms we tried to identify unique fingerprints of protein markers for pregnancies at risk for PE. Some studies have previously reported on the use of maternal serum [2, 4] and plasma [11]. The current study addresses these two important issues. Results from our study indicates that it might be possible to effectively screen for pregnancies at risk for PE using 1st trimester maternal plasma samples and generating proteomic fingerprints by SELDI-TOF MS analysis. Protein profiling is a powerful technique to look at several hundred proteins in a single spectrum [14].

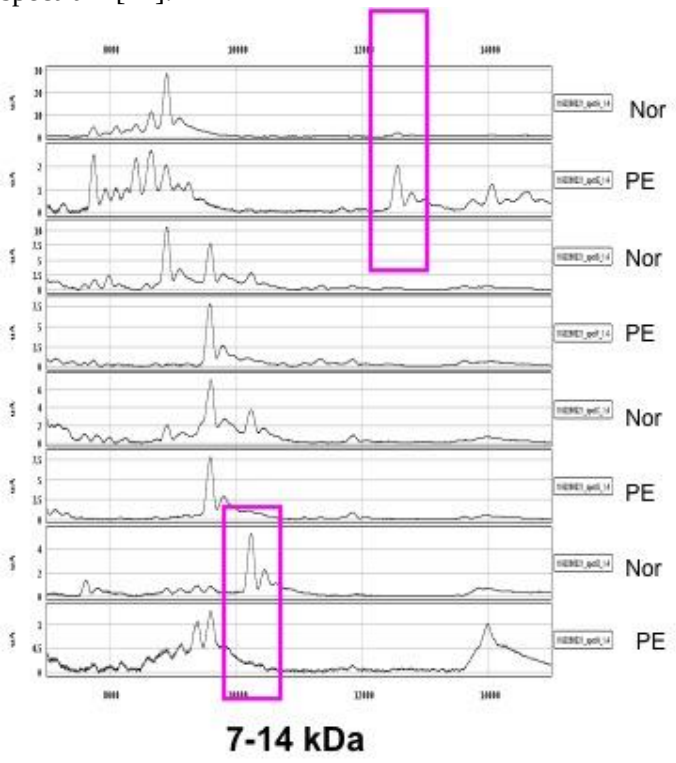

Figure 1: Represent the peak identities corresponding to proteomics features identified using bioinformatics approach to discriminate Normal (Nor) and Pre-eclamptic (PE) cases.

Human serum is known to contain a complex mixture of different kinds of protein and peptides [15], and it is suggested that a success in distinguishing healthy person from a patient could be improved with the identification of unique features in protein profiles [16]. The number of distinct features of spectral information detected in the mass spectrum of a processed sample is a strong indicator of the information content of the signature. Recent studies have focused on using the power of SELDI techniques to study amniotic fluid, cervical vaginal fluid for various disease conditions associated with the developing fetus such as inflammation, infection [17], and neonatal sepsis and also to investigate into the mechanisms of idiopathic preterm birth [14]. The same approach has been used to detect ectopic pregnancies [3] and as well as Down syndrome [4]. Till now this approach has not been explored for prenatal screening in a large scale as well as in clinical settings.

Table 2: Proteomic features identified to discriminate Pre-eclamptic (PE) conditions from normal are based on protein profiling technique. Y is present and N is absent for peak.

Peaks	7.7	8.4	8.9	9.2	10.2	12.6	13.9	22.2
PE	Y	Y	N	Y	Y	Y	Y	Y
Control	N	N	Y	N	Y	N	N	N

Table 3: List of proteins identified in our previous study using quantitative proteomics and SELDI protein profile found in our current study.

S.No	Accession No.	Protein	SELDI Peak MW (KkDa) / Theoretical MW (kDa)
1.	P09382	Galectin-1	13.9 / 14
2.	P00750	Plasminogen	22.2 / 22

In the current analysis, the mass (mw) of the markers ranged between 3 kDa and 60 kDa with majority of the markers in the range less than 22 kDa. The two proteins Galectine (14 kDa) and Plasminogen (22 kDa) indentified in previous study [18] and also in current study indicate that the method is working for SELDI platform as well. These proteins were over expressed in the plasma of PE cases. Galectine is a placental derived protein, which comes under the class of glycoprotein. It plays a crucial role in pregnancy by preventing the attack of maternal immune system to the developing embryo. Plasminogen the inactive precursor of plasmin and is present in most tissue, blood, vessel walls and body fluids.

III. Conclusion

In summary, maternal plasma proteomic profiling with SELDI may prove to be a useful tool for the screening of pregnancies at risk for PE. The accuracy of this approach, however, requires large scale verification

before it can be considered for clinical setup for the screening of the pregnancy.

IV. Material and Methods:

a. Samples

Blood samples for this case-control proteome study were collected prospectively from pregnant women at approximately 12 weeks of gestation first trimester. In a retrospective manner, 6 samples from cases that subsequently developed preeclampsia were matched with 6 samples from pregnancies with normal healthy outcome Table.1 (A) Criteria used for pregnancy with or without preeclampsia for the longitudinal study. (B) Gestational window selected for the current study (n=6) who subsequently developed the preeclampsia. This study was undertaken with the approval of the Institutional Ethical Board of the University Hospital, Basel, Switzerland and written informed consent was required in all instances.

Table 1: (A) Criteria used for pregnancy with or without preeclampsia for the longitudinal study. (B) Gestational window selected for the current study (n=6) who subsequently developed the preeclampsia:

A:

	Control	PE
Maternal age (years)	35.1±1.3	35.1±6.8
Gestational age (weeks)	38.8±0.8	34.9±2.4
Systolic BP (mmHg)	122.5±14.2	188.8±18.6
Diastolic BP (mmHg)	73.6±7.0	111.8±14.1
Proteinuria (dipstick)	Negative	+++

B:

	Control	PE
Gestational age (Weeks)	12.4±1.2	12.3±1.1

b. Sample preparation

As described previously, 9 ml blood was drawn into BD P100 tubes (BD Diagnostics, Franklin Lake, NY, USA), which are specially designed for proteomics experiments, in that the EDTA (Ethylenediaminetetra-acetic acid) and protease inhibitor present in the tube prevent coagulation and stabilize the plasma proteome. Following phlebotomy the samples were centrifuged at 3,000x g for 30 minutes at 10ºC, whereby the plasma was separated from the cellular fraction by aid of a mechanical separator, 100µl aliquots were stored at -80ºC until further use.

c. Plasma protein profiling

Protein profiling was performed using the ProteinChip™ Biomarker System (Ciphergen Biosystems®, Freemont, CA, USA), a SELDI-TOF MS platform. We used H50 (C8 reversed phase/hydrophobic interaction chromatography) ProteinChip™ Array (C573-0065, Ciphergen Biosystems). Samples were applied to H50 protein chip arrays (Ciphergen, Fremont, CA, USA) according to the manufacturer's protocol. Saturated solution of sinapinic acid in 50% acetonitrile, 0.5% trifluoroacetic acid was applied twice to each spot on the array, with air drying between each application. To minimize bias, plasma samples from mothers who developed PE and those with normal outcome were assayed on the same chips. Plasma samples were analyzed using the Protein Biology System 2 SELDI-TOF mass spectrometer (Ciphergen Biosystems). Peptides and proteins below the 60 kDa range were ionized using α-cyano-4-hydroxy-cinnamic acid as the matrix (C300-0002, Ciphergen Biosystems). Known proteins were used for data calibration between experiments (C100-0007, Ciphergen Biosystems), and the mass accuracy was determined daily using the Ciphergen Broad Range molecular weight standards (C100-0001, Ciphergen Biosystems). Chips with plasma samples were analyzed under the following conditions: laser intensity 260V, detector sensitivity 10, mass focus 30 kDa, with molecular mass/charge (m/z) range from 0-60 kDa and mass optimization 3-60 kDa. Data were collected by averaging 65 laser shots per sample. These were exported as raw data (Ciphergen PBSII™ software, Ciphergen Biosystems),), and used without modification for downstream bioinformatics analyses. Spectra were calibrated, baseline subtracted, and normalized. Qualified mass peaks (signal/noise >5; cluster mass window at 0.3%) within the m/z range of 2–60 kDa were selected automatically. Logarithmic transformation was applied to the peak intensity before analysis for biomarker discovery. After biomarker discovery, the quality and intensity readings of the selected peaks were manually reconfirmed from raw spectra.

d. Bioinformatics approach

We analyzed spectra of 6 control and 6 cases that developed PE. Each spectrum had 26,707 mass spectral components. To reduce non-specific peaks especially in the low mass range, we eliminated the first 5,989 components that all corresponded to masses < 3kDa. We manually inspected the remaining part of the spectra and selected 62 apparent peaks in the spectra with the aim to select few that could be used to classify PE cases and separate them from the control healthy cases. These 62 peaks served to generate initial feature set based on which the individual cases were analyzed.

e. Classification process and final selection of features

All classification tasks were done in the same fashion, distinguishing between cases which developed PE and those with normal deliveries, based on the Mahalanobis distance between the groups using the

Lawrence and Solovyev algorithm [19]. We also searched for the minimum number of features and the small portion of the data in the training set, so as to achieve good generalization properties of the linear discriminate model. The process involved separation of each of the groups to the training part and the test part. The model is built using only the training data, and it is tested using the test data.

f. Ethics / Institutional review board approval of research

This study was undertaken with the approval of the Institutional Ethical Board of the University Hospital, Basel, Switzerland and written informed consent was required in all instances.

Authors Contribution: VK & SH has conceived the presented idea. VK has done the sample collection, processing and experiments. NK has performed the computation analysis. IH & OL has provided the clinical support. IH, MC, SH & OL has supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Acknowledgements: We thank Mrs. Vivian Kiefer for her technical assistance and Dr. Andrea for the proof reading of the manuscript.

REFERENCES

1. Rockett, John C. "Genomic and proteomic techniques applied to reproductive biology." (2001): reports4020-1.
2. Kolialexi, Aggeliki, et al. "Mass spectrometry-based proteomics in reproductive medicine." *Mass spectrometry reviews* 27.6 (2008): 624-634.
3. Gerton, G. L., et al. "A serum proteomics approach to the diagnosis of ectopic pregnancy." *Annals of the New York Academy of Sciences* 1022.1 (2004): 306-316.
4. Busch, Anne, et al. "Proteome analysis of maternal serum samples for trisomy 21 pregnancies using ProteinChip arrays and bioinformatics." *Journal of Histochemistry & Cytochemistry* 53.3 (2005): 341-343.
5. Nagalla, Srinivasa R., et al. "Proteomic analysis of maternal serum in down syndrome: identification of novel protein biomarkers." *Journal of proteome research* 6.4 (2007): 1245-1257.
6. Rodland, Karin D. "Proteomics and cancer diagnosis: the potential of mass spectrometry." *Clinical biochemistry* 37.7 (2004): 579-583.
7. Zhang, Zhen, et al. "Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer." *Cancer research* 64.16 (2004): 5882-5890.
8. Otu, Hasan H., et al. "Prediction of diabetic nephropathy using urine proteomic profiling 10 years prior to development of nephropathy." *Diabetes care* 30.3 (2007): 638-643.
9. Meuwis, Marie-Alice, et al. "Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling." *Biochemical pharmacology* 73.9 (2007): 1422-1433.
10. Johann, Donald J., et al. "Clinical proteomics and biomarker discovery." *Annals of the New York Academy of Sciences*1022.1 (2004): 295-305.
11. Kolialexi, Aggeliki, et al. "Application of proteomics for the identification of differentially expressed protein markers for Down syndrome in maternal plasma." *Prenatal diagnosis* 28.8 (2008): 691-698.
12. Krantz, David, et al. "First-trimester maternal dried blood Down syndrome screening marker levels in early pregnancy loss." *Prenatal diagnosis* 26.12 (2006): 1137-1141.
13. Lubec, Gert. "Proteomic evaluation of intermediary metabolism enzyme proteins in fetal Down's syndrome cerebral cortex." *Proteomics* 2 (2002): 1539-1546.
14. Buhimschi, Catalin S., et al. "Proteomic profiling of the amniotic fluid to detect inflammation, infection, and neonatal sepsis." *PLoS medicine* 4.1 (2007): e18.
15. Adkins, Joshua N., et al. "Toward a human blood serum proteome analysis by multidimensional separation coupled with mass spectrometry." *Molecular & Cellular Proteomics*1.12 (2002): 947-955.
16. Wagner, Michael, et al. "Computational protein biomarker prediction: a case study for prostate cancer." *BMC bioinformatics* 5.1 (2004): 26.
17. Pettker, Christian M., et al. "Value of placental microbial evaluation in diagnosing intra-amniotic infection." *Obstetrics & Gynecology* 109.3 (2007): 739-749.
18. Kolla, Varaprasad, et al. "Quantitative proteomic (iTRAQ) analysis of 1st trimester maternal plasma samples in pregnancies at risk for preeclampsia." *BioMed Research International* 305964 (2012): 1-8.
19. Lawrence, Charles B., and Victor V. Solovyev. "Assignment of position-specific error probability to primary DNA sequence data." *Nucleic acids research* 22.7 (1994): 1272-1280.