Applied Medical Research

Review Article

DOI: doi.org/10.47363/AMR/2015(2)113

Preeclampsia at the molecular level

Randa Abdel Kader Mahmoud El-Desouki[1], Fawzia Ahmed Habib[2]

Departments of [1]Biochemistry and Molecular Medicine, [2]Obstetrics and Gynecology, [1,2]Faculty of Medicine, Taibah University, Almadinah Almunawwarah, Saudi Arabia, [1]Faculty of Medicine, Cairo University, Cairo, Egypt

Address for correspondence: Randa Abdel Kader El-Desouki, Clinical Biochemistry and Moleclular Medicine, Taibah University, Almadinah Almunawar, Saudi Arabia. E-mail: randa592003@yahoo.co.uk

Received: January 20, 2015
Accepted: February 09, 2015
Published March 16, 2015

ABSTRACT

In clinical practice, we found despite the promise of some biomarkers in identifying women at increased risk for preeclampsia (PE) is still challenging! PE is a pregnancy-specific syndrome causes substantial maternal and fetal/neonatal morbidity and mortality worldwide. Despite decades of research, the etiology is incompletely understood and hence the ability of clinicians to predict PE prior to the onset of symptoms has not improved significantly. In this review, we will explore potential future areas of research underlying the pathophysiology of PE at the molecular level and potential biomarkers evolving for early prediction and diagnosis hoping to suggest novel therapeutic interventions.

KEY WORDS: Genomic, metabolomic, metagenomic, preeclampsia, proteomic, syncytiotrophoblast microvesicles

INTRODUCTION

Preeclampsia (PE) is a multisystem disorder, affects 2-5% of pregnancies in the Occident, but complicates up to 10% of pregnancies in the developing countries, where emergency care is often inadequate or lacking. It is still the second most common cause of maternal mortality as reported by the confidential enquiry into Maternal and Child Health for the triennium of 2006-2008 [1].

Although the precise etiology of the disease is unclear, accumulating evidence suggests that the disease results from complex interaction between a poorly perfused placenta, because of defective remodeling of the uteroplacental arteries in early pregnancy, and a maternal response to placental derived triggers, which results in a widespread vascular endothelial cell dysfunction [2,3].

PE can have an early- or late- onset starting before or after 34 weeks of gestation respectively; with systolic/diastolic blood pressure ≥160/≥110 mmHg respectively in previously normotensive women, proteinuria >5 g/24 h, hemolysis, elevated liver enzymes, and low platelet counts syndrome (HELLP), and can evolve in eclampsia in the most severe cases [4].

Clinicians have traditionally relied on maternal risk factors, such as increased maternal age, family history, and preexisting diseases, for determining women who are at increased risk. The problem is millions of women worldwide have these factors but do not develop PE [5].

Widespread plasma alterations precede the clinical onset of PE, and numerous candidate biomarkers have been proposed for prediction such as placental hormones, angiogenic factors, and lipids. Until date, none (nor any combination of them) has emerged with the requisite specificity and sensitivity to be of clinical use [5-8].

Uteroplacental Doppler ultrasound has so far been the most widely used for predicting PE. However, it is limited for predicting early and intermediate PE and has a much lower performance for late onset PE [9]. Consequently, clinicians are unable to offer either targeted surveillance or potential preventative therapies especially to nulliparous women at greatest risk [10].

Bioinformatics analysis indicated differentially expressed proteins correlating with several specific cellular processes, which occur during pathological changes of preeclamptic

placenta [11]. It is likely that this line of investigations will open new avenues for potential biomarker discovery for novel diagnostic and preventative measures.

In this review, we will look at novel biomarkers at the molecular level 'omic technologies' "genomic, proteomic, metabolomic, and metagenomic" that have characteristic fingerprints "signature" profiles, hoping it will offer a great prospective for creating new insights into PE pathophysiology.

GENOMICS

A number of gene polymorphisms have been associated with the risk of developing PE, the following are some examples. The finding of an association with maternal carriage of the plasminogen activator inhibitor Type 1 (PAI-1) (2675 4G/5G) promoter polymorphism suggests that plasminogen activation and inhibition pathways may be involved in the etiopathogenesis of PE [12]. Belo *et al*. demonstrated higher plasma levels of PAI-1 in women with PE compared with gestation-matched normotensive pregnant [13]. Excessive release promotes thrombosis, reduces placental perfusion, and triggers the release of factors that culminates the clinical entity of PE [13]. Rahimi *et al*. indicated that methylenetetrahydrofolate reductase C677T polymorphism through effects on triacylglycerol level, lipid peroxidation and oxidative stress might be involved in the pathogenesis of severe PE [14]. However, systematic review and meta-analysis performed by Zhao *et al*. did not support AGTR1 +1166A>C as a susceptibility locus for PE, and they recommended other AGTR1 SNPs to be investigated. In randomized clinical trials, genetic associations might contribute to the complex molecular mechanisms underlying PE, and inform researchers to develop novel interventions [15].

PROTEOMICS

Development of PE involves several different pathophysiological mechanisms, as evidenced by the diversity of the peptides that have been studied to date [16,17].

Placental Mitochondrial Proteome

Nadamuni reported the use of quantitative mitochondrial proteomic analysis to demonstrate that PE is primarily a disorder involving an altered placental mitochondrial proteome [18]. Jiang and Wang have identified decreased expression of some placental mitochondrial proteins such as peroxiredoxin III and HSPA4/HSP70 and increased expression of cytochrome C correlated with the increased caspase 3 in preeclamptic placentae as compared with normals [19]. Shi *et al*. found increased expression of 4 proteins and decreased expression of 22 in preeclamptic placentae compared with normal ones [20]. Bioinformatics analysis revealed critical role of these proteins in apoptosis, fatty acid oxidation, Krebs' cycle, respiratory chain, cellular reactive oxygen species generation and secondary mtDNA mutations [20]. These results suggested that the insufficient energy production in the preeclamptic placenta might prevent invasion of the trophoblast. The degenerative and apoptotic changes in preeclamptic mitochondrial syncytiotrophoblast may be a primary pathologic event or a secondary effect of altered placental oxygenation in PE [18,20].

Urinary Proteomics

Fibrinogen α-chain, collagen α-chain, and uromodulin fragments are among the pregnancy-specific urinary proteomic biomarkers to predict PE at gestational week 28 with good confidence but not reliably at earlier time points from those with an uncomplicated pregnancy. Although there is a limited role for late screening, this set of biomarkers prove a helpful aid for PE diagnosis [21]. Their performance is similar or better than that reported with serum first trimester soluble-like tyrosine kinase-1 and placental growth factor in the second trimester for early-onset PE [22]. Padmanabhan *et al*. have demonstrated that rs13333226 in the promoter region of uromodulin UMOD gene locus is associated with hypertension independently of renal function [23]. Differential urinary expression of UMOD fragments could point toward subclinical vascular and renal damage in the early stage that warrant further investigation [21].

Maternal Blood or Placental Tissue Proteome

Rasanen *et al*. reported that placental and anti-angiogenic proteins are abundant in clinical PE, meanwhile in preclinical PE, proteomic profile is distinct and different from that in clinical PE [24]. In all studies analyzed by Khan *et al*. 192 proteomic biomarkers were catalogued for PE [25]. Another systematic review showed that the pregnant women who are known to have polycystic ovary syndrome (PCOS) were 4 times more likely to develop PE when compared with controls [26]. Proteomic studies in PE and in PCOS discovered a panel of 5 biomarkers including annexin 2, fibrinogen, transferrin, kininogen-1, and peroxiredoxin 2 common for both disorders possibly due to the role of immune regulation/inflammation and antioxidants in their pathogenesis [27,28]. Annexin 2 and fibrinogen are central in regulating fibrinolysis and thrombosis and their altered expression might represent changes in permeability of the vasculature of the various tissues, including ovaries, causing fibrinolysis and abnormal thrombosis in PCOS. Annexin 2 is key physiological receptor for plasminogen on the extracellular surface of endothelial cells, is down-regulated in PE which tilts the coagulation/fibrinolysis balance towards enhanced coagulation and thrombosis [29]. Khan *et al*. postulated that since annexin 2 is down-regulated in both diseases, it could be a strong candidate biomarker for the detection of PE in women with PCOS [25]. Transferrin is expressed significantly in the villous syncytiotrophoblasts and found to be up-regulated in sera of both women compared with those with normal pregnancies. Its upsurge could be explained on the basis of the inflammatory constituent of the two conditions; it is a stress/acute phase response molecule [25]. Kininogen-1 inhibits plasmin and was found to be up-regulated both in women with PE and PCOS in plasma and omental biopsy, respectively [25,30]. In view of the essential role of peroxiredoxin in protecting cells against H_2O_2-induced cell damage and apoptosis emphasizes the role of oxidative stress as an important factor in the development of

PE [29]. It was found to be down-regulated in both conditions in placental and omental biopsy, respectively [25].

Another inter-study comparison identified two proteins with time-dependent changes in expression [11]. Serum complement 4 (C4) was up-regulated at week 12, and down-regulated immediately before delivery; alternatively, the opposite was noticed for apolipoprotein E (apoE) expression. Mao *et al*. mentioned that elevated lipid metabolism and inflammatory/apoptosis parameters suggest a potentially significant role of apoE in PE pathology. During pregnancy, apoE4 promotes atherosclerosis by elevating low density lipoprotein cholesterol levels as well as promoting thrombosis [31]. While apoE has been pursued as promising clinical biomarkers; C4 requires further studies using larger patient cohorts and more targeted quantitative approaches [11]. However, Atkinson *et al*. suggested that apoE levels are unaffected by PE, but polymorphisms result in misrepresentation of glycosylated isoforms in affected women [32]. This is a possible explanation for the conflicted reports of apoE levels in women with PE as these studies could detect different isoforms [32]. In a population with Kurdish ethnic background, Ahmadi *et al*. indicated a protective role for apoE2 allele against severe PE because of its high antioxidant capacity [33].

The proteoglycan Lumican, the glycoprotein Vitronectin and CLIC3 are detected as up-regulated in placenta and serum studies, indicating a possible link between production by the placenta and deposition into serum [34]. Epiney *et al*. acknowledged that syncytial trophoblasts, not cytotrophoblast directly exchange materials with the maternal circulation, these different cell types may explain why there is differential significance between serum studies and the cytotrophoblast sectromone [35].

A total of 171 proteins were differentially identified in human placental proteome profile between control and preeclamptic placentas, of which 147 were down-regulated while 24 were up-regulated [36]. Through comparative proteome analysis, Wang *et al*. constructed a protein expression profile, including circulating soluble form of endoglin (sEng) an anti-angiogenic factor, ceruloplasmin (CP), superoxide dismutase (SOD), transforming growth factor (TGF-β), etc., which play key roles in the incidence and development of PE [36]. Immunolocalization studies characterize matrix metalloproteinases-14 to contribute sEng shedding in severe early-onset preeclamptic placenta. Plasma levels of sEng is increased in women with PE correlated with disease severity, and is a promising accurate marker allowing for early diagnosis, and preventive therapy [37,38]. It blocks the effects of TGF-β on endothelial nitric oxide-mediated vasorelaxation through binding with high affinity to BMP9, which stimulates secretion of the vascoconstrictor endothelin-1 from endothelial cells. It remains to be determined if scavenging of circulating BMP9 by sEng is important in PE and regulation of hypertension [39]. On the contrary, the syncytial CP induced by severe PE, is an important endogenous cellular program to mitigate the damaging effects of subsequent reperfusion injury by enhancing ferro-oxidative activity oxidizing excess Fe^{2+} to the less toxic Fe^{3+} form [40]. Down-regulation of SOD, uridine diphosphate-glucose 6-dehydrogenase and prenylcysteine oxidase 1 proteins are associated with oxidative stress explaining the maternal vascular disease and placental dysfunction [41]. In addition, 21 immunoregulatory proteins were down expressed in PE patients, including interleukin-27 subunit beta, and hemoglobin subunit zeta (about 4.9 fold) [36,42]. Therefore, disruption of metabolic pathways in placenta is of particular relevance to the incidence and development of PE [36].

METABOLOMIC/LIPIDOMIC ANALYSIS

First-trimester serum glycerol, carnitine, methylhistidine, and acetone appeared to be the most important metabolites for distinguishing late-onset preeclampetic patients compared to normal. Methylhistidine was also combined with glycine and carnitine to form a biochemistry-only algorithm. Glycerol, acetate, trimethylamine, and succinate appeared to be the most important metabolites for distinguishing the 2 types of PE [43]. Based on the metabolomic and proteomic analysis performed by the respected investigators [11,16,17,31,44], a picture of a central disturbance of lipid metabolism in late-onset PE emerges. There is a well-documented relationship between maternal obesity and increased risk of late-onset PE. Inflammatory aspect characterizes the lipotoxicity of adipose tissues leads to maternal endothelial dysfunction, and decreases trophoblastic invasion [45]. Glycerol forms the backbone of lipids. Carnitine is responsible for the transport of fatty acids for energy metabolism, and prevents lipid peroxidation. It is synthesized in the liver and kidneys, which are both significantly affected in PE [43]. There was little overlap between the metabolites of significant diagnostic value in the other publications, which could be due to use of different metabolomic platforms for detection or whether the studies focused primarily on early- or late onset PE [10,44,45]. In the study by Odibo *et al*. the significant metabolites identified were primarily amino acids [44]. The diagnostic accuracy of the metabolites combined with race and/or weight appears higher than that reported for the widely available traditional clinical markers used [43]. The finding of a consistent plasma discriminatory metabolite signature as early as 15 weeks' gestation preceding the onset of PE, would offer the tantalizing promise of a robust presymptomatic screening test that could provide insight into disease pathogenesis [10,43].

Korkes *et al*. and other investigators found significant increase of glycerophospho -serine (PS) and -choline (PC) and decrease of -ethanolamine (PEt), in plasma and placenta samples of women with early-onset PE compared to healthy pregnant [46-48]. PS representing the major lipid constituent of cell membranes and lipoproteins, acts as the signaling molecules involved in the processes of oxidative stress, apoptosis and coagulation, which are all exacerbated in PE [49]. PC is the precursors of several lipid second messengers and their increased levels have been associated with increased cell proliferation [50]. The reduction of PEt in the endoplasmic reticulum is associated with arachidonic acid release, which is the precursor of thromboxanes and prostacyclins that act in opposing mechanisms [51]. Sphingomyelin was found only in plasma samples of patients with PE [46]. It is involved in processes of

endothelial dysfunction, increased production of angiotensin II and thromboxane A2 hence, hypertensive disorders [52] which could explain its exclusively occurrence in patients with PE [46].

Korkes *et al*. found flavonoids in plasma and placenta samples of PE and normal groups and macrolides polyketides-lactone-PK04 in placenta samples of PE group only [46]. These lipid groups are not present in mammalian lipid composition, and probably their presence are derived from the diet for the former and from bacteria or fungi for the latter. They found an increase and a decrease of the flavonoids levels in plasma and placenta samples respectively in patients with PE when compared to controls [46]. Evidences support flavonoids as antioxidants and to operate in the signaling pathways both in promotion and inhibition of apoptotic processes [53]. Rapamycin "Sirolimus" is an important polyketide with many biological activities, including immunosuppressive, apoptotic activities and has been associated with the development or exacerbation of proteinuria [54]. It inhibits proliferation by interfering with the function of its mammalian target "mTOR signaling pathway," which has key role as a regulator of invasive trophoblast differentiation/proliferation. In the mature placenta mTOR is expressed at the mRNA level, however, its cellular localization and the functional role after implantation and early placental development remains unknown [55]. Korkes *et al*. recommended further studies to clarify if lipid changes are specific as a cause or consequence of PE [46].

Microbiome/Metagenomic of Gut, Placenta, and Vagina

There is evidence that maternal microbiome complex changes naturally during pregnancy, increasing potentially pathogenic Gram-negative proteobacteria; this is likely related to reduced gastrointestinal motility caused by increased progesterone level [56]. Roger and Rosano recognized that women residing in low and middle income countries have greater environmental exposure to Gram-negative bacteria due to widespread fecal contamination of drinking water [57]. Koren *et al*. found that through pyrosequencing of fecal bacteria in 91 pregnant women from first to third trimester (T1, T3), the microbiota in T1 was similar to non-pregnant controls, by T3 there was a significant increase of proteobacteria, actinobacteria, and clostridia composition, so remarkable that it resembled a dysbiosis [56]. In pregnant lipopolysaccharide (LPS)-exposed rats, TNF-α mediated inflammation that resulted in deficient trophoblastic invasion, increased spiral artery resistance index, placental nitrosative stress, increased maternal mean arterial pressure, renal structural alterations, and significant elevation of protein: Creatinine ratios which are all characteristic of severe PE [58].

Aagaard *et al*. characterized a unique placental microbiome niche through comparative 16S ribosomal DNA-based and whole-genome shotgun "metagenomic studies" composed of nonpathogenic commensal microbiota such as Bacteroidetes phyla, which were most akin to the human oral microbiome from non-pregnant controls [59].

Pregnancy is characterized by a stable Lactobacillus dominated community species [60,61], which actively protect themselves and the vaginal environment from invaders by the production of lactic acid, which acidifies the vaginal pH as well as the production of H_2O_2 which prevents ascending infection [62]. A healthy vaginal microbiome may be a stronghold against potential microbial invaders [63].

Vaginal and placental microbiomes are also altered in pregnancy that together with gut microbiome could contribute to pregnancy complications [62,64]. 16S based operational taxonomic unit analyses revealed associations of the placental microbiome with a remote history of antenatal infection, such as urinary tract infection in the first trimester, as well as with preterm birth <37 weeks [59].

NEW INSIGHTS INTO PE PATHOPHYSIOLOGY

Placental Syncytiotrophoblast Microvesicles (STBM)

While shedding of apoptotic corpuscular structures from the syncytiotrophoblast is part of the normal turnover of villous trophoblast throughout pregnancy [65]. In PE this process is dysregulated by the release of placental STBM particularly in the 2nd and 3rd trimester, which provoke the systemic inflammatory response and endothelial damage of the mother [66]. Over 400 proteins identified in the STBM samples, 25 implicated in immune response, coagulation, oxidative stress, apoptosis as well as lipid metabolism pathways [49] were found to be differentially expressed in PE compared to healthy pregnant controls [67]. Their expressions possibly depict the syncytiotrophoblast response at the maternal-fetal interface to the underlying pathology [67]. Anti-apoptosis annexin A4 was up-regulated to counteract pro-inflammatory molecules such that homeostasis is achieved [68]. Increased expression of glyceraldehyde-3-phosphate dehydrogenase an anaerobic enzyme in glycolysis could be associated with the oxidative stress that exists in PE [69]. Down-regulation of integrin may be associated with shallow trophoblast invasion and defective placental vasculature in PE [67,70]. While, down-regulation of histones may be suggestive of increased in DNA damage/defective repair, and raised inflammatory response in adverse PE [67,71]. Baig *et al*. supported the currently emerging role of STBM as contributors to the pro-inflammatory state of PE [67].

Placental Danger Signal or Alarmin

STBM proteins also include endogenous danger molecules or alarmins such as extracellular free actins, tubulins, and heat shock proteins, which have intensely pro-inflammatory properties [67]. Normally, serum placental protein 13 (PP13) slowly increases during pregnancy and shows double-to-triple values close to delivery, after which it disappears from maternal blood. In contrast, in the first trimester at 5-7 weeks of gestation in women developing PE later in pregnancy, it is significantly lower than normal. The sharper the increase of PP13 from first to third trimester, the more severe the anticipated PE symptoms [65]. Colocalization of PP13 with annexin 2, PLAP and CD71 in the syncytiotrophoblast brush border membrane of control placentas, suggested an equal distribution of PP13 in lipid raft and nonlipid raft regions [72]. However, Balogh *et al*. observed that the apical,

nonlipid raft localization of PP13 in the syncytiotrophoblast brush border is reduced, and its association with the juxtamembrane cortical actin network and lipid raft domains is enhanced in preterm PE and HELLP syndrome. Possibly, this leads to its elevation in the maternal circulation through facilitating secretion and/or shedding of STBM [73]. These findings were consistent with reports showing similar changes in placental pathology and global transcriptome in both disorders [74].

Women with low PP13 levels in the first trimester may lack the necessary amount to enable immune tolerance and to prepare the maternal vasculature for the increased blood flow needed to supply the fetus during the second half of pregnancy. Its increased release from the aponecrotic trophoblast upon ischemic stress may contribute to the exaggerated activation of the maternal immune system in preterm PE and HELLP syndrome [73]. They proposed that PP13 can function as an endogenous danger "alarmin" of the syncytiotrophoblast [73]. Replenishing PP13 early in pregnancy for keeping its levels within a 'therapeutic window' is a new direction to transfer individualized risk to personalized prevention requires further safety studies, considering that not all patients with low PP13 will develop PE [75].

INTRA-ABDOMINAL HYPERTENSION IN PREGNANCY

In 2011, Sugerman published a hypothesis that PE is a venous disease secondary to increased intra-abdominal pressure (IAP) in pregnancy >12 mmHg [76], that when sustained or increasing, leads to hemodynamic shifts, intestinal ischemia-reperfusion injury causing mucosal epithelial injury, translocation of LPS endotoxin to the liver, systemic cytotoxic immune response, multi-organ dysfunction, and poly-compartment syndrome [77-79].

Evidence based sequence demonstrated that the threshold for LPS translocation depends on the magnitude of IAP, the intestinal microbiome complex, and the degree of intestinal permeability [79]. Sawchuck and Wittmann speculated that delivery cures PE through the mechanism of abdominal decompression [79].

Zonulin (Zot) protein is a biomarker for gut permeability [80]. Epithelial integrity can be compromised by Zot dysregulation due to exposure to pathogenic bacteria leading to loss of intestinal barrier function [81]. Studies showed that the presence of lactobacillus plantarum and probiotic escherichia coli Nissle 1917 in the small intestine mediates Zot upregulation and confers protection of the epithelial barrier [82]. Zhang *et al*. suggested that alterations of gut permeability may play a role in the pathophysiology of PCOS [83]. The increase in serum Zot levels and its correlation with insulin resistance and severity of menstrual disorders allowing it to be a useful biomarker for both risk stratification and therapeutic outcomes in PCOS women [83].

Little is known about changes in intestinal permeability during normal pregnancy, and it appears to never have been thoroughly investigated [83]. The high incidence of PE in pregnant women with PCOS has to be considered here. Sawchuck and Wittmann suggested that the bowel has truly been the forgotten organ during pregnancy, and its potential role in the etiology of PE warrants investigation [79].

PE MISFOLDOME

Buhimschi *et al*. showed that PE shares pathophysiologic features with recognized protein misfolding disorders. These features include urine congophilia, affinity for conformational state-dependent antibodies, and placental up-regulation of prototype proteolytic enzymes involved in amyloid precursor protein processing. The urine congophilic material includes proteoforms of CP, immunoglobulin free light chains, SERPINA1, albumin, interferon-inducible protein 6-16, and Alzheimer's β-amyloid, their assessment carries diagnostic and prognostic potential for PE. Conformational state-dependent antibodies usage demonstrate the presence of generic pre-fibrillar oligomers and protofibrils, which vary in quantitative and qualitative representation with PE severity [84].

The role of hypoxia in inducing prion-like protein aggregation remains at present controversial, but cannot be excluded [85,86]. The presence of β-amyloid aggregates in preeclamptic placentas suggest that this condition could join the growing list of protein conformational disorders however, further investigations are necessary [84,86].

RETICULATED PLATELETS (RP)

RPs are immature platelets, newly released from the bone marrow into the circulation, and are more active in thrombus formation. PE is associated with increased platelet activation involved in its pathogenesis not only promoting coagulation, but also as an important inflammatory mediator [87]. However, PE is frequently associated with thrombocytopenia due to platelet consumption being trapped in the plug. In response, young platelets would be released into the peripheral circulation. Everett *et al*. demonstrated direct evidence that increased circulating RPs were useful for monitoring PE, which may reflect increased platelet consumption during the evolution of placenta thrombosis or contribute to PE pathology [87]. However, Dusse and Freitas recommended additional randomized and well-controlled clinical studies to clearly establish the significance of circulating RPs [88].

PROBIOTICS/PREBIOTICS

Prenatal probiotics and prebiotics supplementation significantly regulate the unbalanced microflora composition, reduce the incidence of bacterial vaginosis, increase colonization with vaginal and intestinal *Lactobacillus rhamnosus*, reduce maternal fasting glucose, incidence of gestational diabetes, levels of C-reactive protein, and PE rates [89,90]. They were also associated with significantly higher counts of Bifidobacterium and Lactococcus lactis in maternal intestine and in neonatal stool [89,90]. Ekambaram *et al*. demonstrated that a diet rich in

yeast species Monascus purpureus and Saccharomyces cerevisiae could serve as a good natural antioxidant source and probiotic supplement to alleviate the stress status of preeclamptic patients and their babies. They had significant catalase activity, inhibited formation of lipid peroxide and nitrite/nitrate in cord blood red blood count of preeclamptic and normotensive subjects [91].

However, in obese women, Lindsay *et al*. reported that treatment of either a daily probiotic or a placebo capsule from 24th to 28th week during pregnancy did not influence maternal fasting glucose, metabolic profile, or pregnancy outcomes [92]. Also, Parrish *et al*. stated that initiation of antioxidant/phytonutrient supplementation in the first trimester continued throughout the gestation did not decrease rates of PE [93]. Gomez Arango *et al*. recommended large, well-designed randomized controlled clinical trials along with metagenomic analysis to establish the role of probiotics in adverse pregnancy and infancy outcomes [64].

CONCLUSION

Although improvements in obstetric and neonatal care have led to a reduction in morbidity and mortality of PE. Our ability to predict this devastating condition has not improved significantly, clinicians currently rely on PE secondary prevention. The majority of women are only diagnosed once they have developed the full blown manifestations, by which time treatment options are limited. An increased understanding of the molecular mechanisms underlying PE has led to several potential areas of investigation. It is hoped that combining genomic, proteomic, metabolomic, metagenomic, etc. biological approaches will give us more comprehensive understanding about PE. Mechanistically, they will relate the genome to the expressed phenotype to develop biomarkers with high enough predictive and prognostic information, to be translated into clinical practice in the near future.

REFERENCES

1. Cantwell R, Clutton-Brock T, Cooper G, Dawson A, Drife J, Garrod D, *et al*. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom. BJOG 2011;118 Suppl 1:1-203.
2. Grill S, Rusterholz C, Zanetti-Dällenbach R, Tercanli S, Holzgreve W, Hahn S, *et al*. Potential markers of preeclampsia – A review. Reprod Biol Endocrinol 2009;7:70.
3. de Oliveira LG, Karumanchi A, Sass N. [Preeclampsia: Oxidative stress, inflammation and endothelial dysfunction]. Rev Bras Ginecol Obstet 2010;32:609-16.
4. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. Semin Perinatol 2012;36:56-9.
5. Rana S, Cerdeira AS, Wenger J, Salahuddin S, Lim KH, Ralston SJ, *et al*. Plasma concentrations of soluble endoglin versus standard evaluation in patients with suspected preeclampsia. PLoS One 2012;7:e48259.
6. Myers JE, Tuytten R, Thomas G, Laroy W, Kas K, Vanpoucke G, *et al*. Integrated proteomics pipeline yields novel biomarkers for predicting preeclampsia. Hypertension 2013;61:1281-8.
7. Myers JE, Kenny LC, McCowan LM, Chan EH, Dekker GA, Poston L, *et al*. Authors' reply: Angiogenic factors combined with clinical risk factors to predict preterm pre-eclampsia in nulliparous women: A predictive test accuracy study. BJOG 2014;121:507.
8. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, *et al*. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: The Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension 2014;64:644-52.
9. Huppertz B, Meiri H, Gizurarson S, Osol G, Sammar M. Placental protein 13 (PP13): A new biological target shifting individualized risk assessment to personalized drug design combating pre-eclampsia. Hum Reprod Update 2013;19:391-405.
10. Kenny LC, Broadhurst DI, Dunn W, Brown M, North RA, McCowan L, *et al*. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension 2010;56:741-9.
11. Wong F, Cox B. Proteomics analysis of preeclampsia. A systematic review of maternal and fetal compartments. J Proteomics Bioinform 2014;S10:001.
12. Morgan JA, Bombell S, McGuire W. Association of plasminogen activator inhibitor-type 1 (-675 4G/5G) polymorphism with pre-eclampsia: Systematic review. PLoS One 2013;8:e56907.
13. Belo L, Santos-Silva A, Rumley A, Lowe G, Pereira-Leite L, Quintanilha A, *et al*. Elevated tissue plasminogen activator as a potential marker of endothelial dysfunction in pre-eclampsia: Correlation with proteinuria. BJOG 2002;109:1250-5.
14. Rahimi Z, Malek-Khosravi S, Rahimi Z, Jalilvand F, Parsian A. MTHFR C677T and eNOS G894T variants in preeclamptic women: Contribution to lipid peroxidation and oxidative stress. Clin Biochem 2013;46:143-7.
15. Zhao L, Dewan AT, Bracken MB. Association of maternal AGTR1 polymorphisms and preeclampsia: A systematic review and meta-analysis. J Matern Fetal Neonatal Med 2012;25:2676-80.
16. Park J, Cha DH, Lee SJ, Kim YN, Kim YH, Kim KP. Discovery of the serum biomarker proteins in severe preeclampsia by proteomic analysis. Exp Mol Med 2011;43:427-35.
17. Kolla V, Jenö P, Moes S, Lapaire O, Hoesli I, Hahn S. Quantitative proteomic (iTRAQ) analysis of 1st trimester maternal plasma samples in pregnancies at risk for preeclampsia. J Biomed Biotechnol 2012;2012:305964.
18. Nadamuni S. Proteomic analysis of mitochondria unravels the pathophysiology of preeclampsia. Proteomics Accel Sci 2013;1-3. Available from http://acceleratingscience.com [Last accessed on 2014 Oct 9].
19. Jiang Y, Wang X. Comparative mitochondrial proteomics: Perspective in human diseases. J Hematol Oncol 2012;5:11.
20. Shi Z, Long W, Zhao C, Guo X, Shen R, Ding H. Comparative proteomics analysis suggests that placental mitochondria are involved in the development of pre-eclampsia. PLoS One 2013;8:e64351.
21. Carty DM, Siwy J, Brennand JE, Zürbig P, Mullen W, Franke J, *et al*. Urinary proteomics for prediction of preeclampsia. Hypertension 2011;57:561-9.
22. Sunderji S, Gaziano E, Wothe D, Rogers LC, Sibai B, Karumanchi SA, *et al*. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: A prospective clinical study. Am J Obstet Gynecol 2010;202:40.e1-7.
23. Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, *et al*. Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet 2010;6:e1001177.
24. Rasanen J, Girsen A, Lu X, Lapidus JA, Standley M, Reddy A, *et al*. Comprehensive maternal serum proteomic profiles of preclinical and clinical preeclampsia. J Proteome Res 2010;9:4274-81.
25. Khan GH, Galazis N, Docheva N, Layfield R, Atiomo W. Overlap of proteomics biomarkers between women with pre-eclampsia and PCOS: A systematic review and biomarker database integration. Hum Reprod 2015;30:133-48.
26. Kjerulff LE, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: A metaanalysis. Am J Obstet Gynecol 2011;204:558.e1-6.
27. Yun SH, Moon YS, Sohn SH, Jang IS. Effects of cyclic heat stress or vitamin C supplementation during cyclic heat stress on HSP70, inflammatory cytokines, and the antioxidant defense system in Sprague Dawley rats. Exp Anim 2012;61:543-53.
28. Cubedo J, Ramaiola I, Padró T, Martin-Yuste V, Sabate-Tenas M, Badimon L. High-molecular-weight kininogen and the intrinsic coagulation pathway in patients with de novo acute myocardial infarction. Thromb Haemost 2013;110:1121-34.
29. Al-ofi E, Coffelt SB, Anumba DO. Fibrinogen, an endogenous ligand

of Toll-like receptor 4, activates monocytes in pre-eclamptic patients. J Reprod Immunol 2014;103:23-8.
30. Wong MK, Takei Y. Lack of plasma kallikrein-kinin system cascade in teleosts. PLoS One 2013;8:e81057.
31. Mao L, Zhou Q, Zhou S, Wilbur RR, Li X. Roles of apolipoprotein E (ApoE) and inducible nitric oxide synthase (iNOS) in inflammation and apoptosis in preeclampsia pathogenesis and progression. PLoS One 2013;8:e58168.
32. Atkinson KR, Blumenstein M, Black MA, Wu SH, Kasabov N, Taylor RS, *et al*. An altered pattern of circulating apolipoprotein E3 isoforms is implicated in preeclampsia. J Lipid Res 2009;50:71-80.
33. Ahmadi R, Rahimi Z, Vaisi-Raygani A, Kiani A, Jalilian N, Rahimi Z. Apolipoprotein E genotypes, lipid peroxidation, and antioxidant status among mild and severe preeclamptic women from western Iran: Protective role of apolipoprotein e2 allele in severe preeclampsia. Hypertens Pregnancy 2012;31:405-18.
34. Murthi P, Stevenson JL, Money TT, Borg AJ, Brennecke SP, Gude NM. Placental CLIC3 is increased in fetal growth restriction and pre-eclampsia affected human pregnancies. Placenta 2012;33:741-4.
35. Epiney M, Ribaux P, Arboit P, Irion O, Cohen M. Comparative analysis of secreted proteins from normal and preeclamptic trophoblastic cells using proteomic approaches. J Proteomics 2012;75:1771-7.
36. Wang F, Shi Z, Wang P, You W, Liang G. Comparative proteome profile of human placenta from normal and preeclamptic pregnancies. PLoS One 2013;8:e78025.
37. Kaitu'u-Lino TJ, Palmer KR, Whitehead CL, Williams E, Lappas M, Tong S. MMP-14 is expressed in preeclamptic placentas and mediates release of soluble endoglin. Am J Pathol 2012;180:888-94.
38. Kaitu'u-Lino TJ, Tuohey L, Ye L, Palmer K, Skubisz M, Tong S. MT-MMPs in pre-eclamptic placenta: Relationship to soluble endoglin production. Placenta 2013;34:168-73.
39. Gregory AL, Xu G, Sotov V, Letarte M. Review: The enigmatic role of endoglin in the placenta. Placenta 2014;35 Suppl:S93-9.
40. Romanowicz L, Bankowski E, Jaworski S. Stimulation of glycosaminoglycan biosynthesis by umbilical cord serum of newborns delivered by mothers with EPH gestosis (preeclampsia). Pathobiology 2000;68:264-9.
41. Heazell AE, Brown M, Worton SA, Dunn WB. Review: The effects of oxygen on normal and pre-eclamptic placental tissue – Insights from metabolomics. Placenta 2011;32 Suppl 2:S119-24.
42. Zhang Z, Gao Y, Zhang L, Jia L, Wang P, Zhang L, *et al*. Alterations of IL-6, IL-6R and gp130 in early and late onset severe preeclampsia. Hypertens Pregnancy 2013;32:270-80.
43. Bahado-Singh RO, Akolekar R, Mandal R, Dong E, Xia J, Kruger M, *et al*. First-trimester metabolomic detection of late-onset preeclampsia. Am J Obstet Gynecol 2013;208:58.e1-7.
44. Odibo AO, Goetzinger KR, Odibo L, Cahill AG, Macones GA, Nelson DM, *et al*. First-trimester prediction of preeclampsia using metabolomic biomarkers: A discovery phase study. Prenat Diagn 2011;31:990-4.
45. Jarvie E, Hauguel-de-Mouzon S, Nelson SM, Sattar N, Catalano PM, Freeman DJ. Lipotoxicity in obese pregnancy and its potential role in adverse pregnancy outcome and obesity in the offspring. Clin Sci (Lond) 2010;119:123-9.
46. Korkes HA, Sass N, Moron AF, Câmara NO, Bonetti T, Cerdeira AS, *et al*. Lipidomic assessment of plasma and placenta of women with early-onset preeclampsia. PLoS One 2014;9:e110747.
47. De Oliveira L, Câmara NO, Bonetti T, Lo Turco EG, Bertolla RP, Moron AF, *et al*. Lipid fingerprinting in women with early-onset preeclampsia: A first look. Clin Biochem 2012;45:852-5.
48. Baig S, Lim JY, Fernandis AZ, Wenk MR, Kale A, Su LL, *et al*. Lipidomic analysis of human placental syncytiotrophoblast microvesicles in adverse pregnancy outcomes. Placenta 2013;34:436-42.
49. Leventis PA, Grinstein S. The distribution and function of phosphatidylserine in cellular membranes. Annu Rev Biophys 2010;39:407-27.
50. Hernando E, Sarmentero-Estrada J, Koppie T, Belda-Iniesta C, Ramírez de Molina V, Cejas P, *et al*. A critical role for choline kinase-alpha in the aggressiveness of bladder carcinomas. Oncogene 2009;28:2425-35.
51. Meikle PJ, Christopher MJ. Lipidomics is providing new insight into the metabolic syndrome and its sequelae. Curr Opin Lipidol 2011;22:210-5.
52. Spijkers LJ, van den Akker RF, Janssen BJ, Debets JJ, De Mey JG, Stroes ES, *et al*. Hypertension is associated with marked alterations in sphingolipid biology: A potential role for ceramide. PLoS One 2011;6:e21817.
53. Akhlaghi M, Bandy B. Preconditioning and acute effects of flavonoids in protecting cardiomyocytes from oxidative cell death. Oxid Med Cell Longev 2012;2012:782321.
54. Ko HT, Yin JL, Wyburn K, Wu H, Eris JM, Hambly BD, *et al*. Sirolimus reduces vasculopathy but exacerbates proteinuria in association with inhibition of VEGF and VEGFR in a rat kidney model of chronic allograft dysfunction. Nephrol Dial Transplant 2013;28:327-36.
55. Dowling RJ, Topisirovic I, Fonseca BD, Sonenberg N. Dissecting the role of mTOR: Lessons from mTOR inhibitors. Biochim Biophys Acta 2010;1804:433-9.
56. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, *et al*. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470-80.
57. Rogler G, Rosano G. The heart and the gut. Eur Heart J 2014;35:426-30.
58. Cotechini T, Komisarenko M, Sperou A, Macdonald-Goodfellow S, Adams MA, Graham CH. Inflammation in rat pregnancy inhibits spiral artery remodeling leading to fetal growth restriction and features of preeclampsia. J Exp Med 2014;211:165-79.
59. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, *et al*. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One 2012;7:e36466.
60. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med 2014;6:237ra65.
61. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, *et al*. Correction: The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome 2014;2:10.
62. Reid G. Modulating the vaginal microbiome: The need for a bridge between science and practice. Semin Reprod Med 2014;32:28-34.
63. Walther-António MR, Jeraldo P, Berg Miller ME, Yeoman CJ, Nelson KE, Wilson BA, *et al*. Pregnancy's stronghold on the vaginal microbiome. PLoS One 2014;9:e98514.
64. Gomez Arango LF, Barrett HL, Callaway LK, Nitert MD. Probiotics and pregnancy. Curr Diab Rep 2015;15:567.
65. Huppertz B, Sammar M, Chefetz I, Neumaier-Wagner P, Bartz C, Meiri H. Longitudinal determination of serum placental protein 13 during development of preeclampsia. Fetal Diagn Ther 2008;24:230-6.
66. Southcombe J, Tannetta D, Redman C, Sargent I. The immunomodulatory role of syncytiotrophoblast microvesicles. PLoS One 2011;6:e20245.
67. Baig S, Kothandaraman N, Manikandan J, Rong L, Ee KH, Hill J, *et al*. Proteomic analysis of human placental syncytiotrophoblast microvesicles in preeclampsia. Clin Proteomics 2014;11:40.
68. El Kebir D, József L, Filep JG. Opposing regulation of neutrophil apoptosis through the formyl peptide receptor-like 1/lipoxin A4 receptor: Implications for resolution of inflammation. J Leukoc Biol 2008;84:600-6.
69. Zala D, Hinckelmann MV, Yu H, Lyra da Cunha MM, Liot G, Cordelières FP, *et al*. Vesicular glycolysis provides on-board energy for fast axonal transport. Cell 2013;152:479-91.
70. Huppertz B, Berghold VM, Kawaguchi R, Gauster M. A variety of opportunities for immune interactions during trophoblast development and invasion. Am J Reprod Immunol 2012;67:349-57.
71. Liang D, Burkhart SL, Singh RK, Kabbaj MH, Gunjan A. Histone dosage regulates DNA damage sensitivity in a checkpoint-independent manner by the homologous recombination pathway. Nucleic Acids Res 2012;40:9604-20.
72. Godoy V, Riquelme G. Distinct lipid rafts in subdomains from human placental apical syncytiotrophoblast membranes. J Membr Biol 2008;224:21-31.
73. Balogh A, Pozsgay J, Matkó J, Dong Z, Kim CJ, Várkonyi T, *et al*. Placental protein 13 (PP13/galectin-13) undergoes lipid raft-associated subcellular redistribution in the syncytiotrophoblast in preterm preeclampsia and HELLP syndrome. Am J Obstet Gynecol 2011;205:156.e1-14.
74. Várkonyi T, Nagy B, Füle T, Tarca AL, Karászi K, Schönléber J, *et al*. Microarray profiling reveals that placental transcriptomes of early-onset HELLP syndrome and preeclampsia are similar. Placenta 2011;32 Suppl:S21-9.
75. Huppertz B, Meiri H, Gizurarson S, Osol G, Sammar M. Placental protein 13 (PP13): A new biological target shifting individualized risk

assessment to personalized drug design combating pre-eclampsia. Hum Reprod Update 2013;19:391-405.
76. Sugerman HJ. Hypothesis: Preeclampsia is a venous disease secondary to an increased intra-abdominal pressure. Med Hypotheses 2011;77:841-9.
77. Kirkpatrick AW, Roberts DJ, De Waele J, Jaeschke R, Malbrain ML, De Keulenaer B, *et al*. Intra-abdominal hypertension and the abdominal compartment syndrome: Updated consensus definitions and clinical practice guidelines from the World Society of the Abdominal Compartment Syndrome. Intensive Care Med 2013;39:1190-206.
78. Malbrain ML, De Laet IE, De Waele JJ, Kirkpatrick AW. Intra-abdominal hypertension: Definitions, monitoring, interpretation and management. Best Pract Res Clin Anaesthesiol 2013;27:249-70.
79. Sawchuck DJ, Wittmann BK. Pre-eclampsia renamed and reframed: Intra-abdominal hypertension in pregnancy. Med Hypotheses 2014;83:619-32.
80. Zak-Golab A, Kocelak P, Aptekorz M, Zientara M, Juszczyk L, Martirosian G, *et al*. Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects. Int J Endocrinol 2013;2013:674106.
81. Fasano A. Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity, and cancer. Physiol Rev 2011;91:151-75.
82. Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJ, *et al*. Regulation of human epithelial tight junction proteins by Lactobacillus plantarum *in vivo* and protective effects on the epithelial barrier. Am J Physiol Gastrointest Liver Physiol 2010;298:G851-9.
83. Zhang D, Zhang L, Yue F, Zheng Y, Russell R. Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation. Eur J Endocrinol 2015;172:29-36.
84. Buhimschi IA, Nayeri UA, Zhao G, Shook LL, Pensalfini A, Funai EF, *et al*. Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia. Sci Transl Med 2014;6:245ra92.
85. Holmes DL, Lancaster AK, Lindquist S, Halfmann R. Heritable remodeling of yeast multicellularity by an environmentally responsive prion. Cell 2013;153:153-65.
86. Morinet F, Rozenberg F. Preeclampsia: Hypoxia and/or misfolding. Sci Transl Med 2014;6:245-92.
87. Everett TR, Garner SF, Lees CC, Goodall AH. Immature platelet fraction analysis demonstrates a difference in thrombopoiesis between normotensive and preeclamptic pregnancies. Thromb Haemost 2014;111:1177-9.
88. Dusse LM, Freitas LG. Clinical applicability of reticulated platelets. Clin Chim Acta 2015;439:143-7.
89. VandeVusse L, Hanson L, Safdar N. Perinatal outcomes of prenatal probiotic and prebiotic administration: An integrative review. J Perinat Neonatal Nurs 2013;27:288-301.
90. Lindsay KL, Walsh CA, Brennan L, McAuliffe FM. Probiotics in pregnancy and maternal outcomes: A systematic review. J Matern Fetal Neonatal Med 2013;26:772-8.
91. Ekambaram P, Jayachandran T, Venkatraman U, Leonard S. Preeclamptic cord blood hemolysis and the effect of Monascus purpureus and Saccharomyces cerevisiae in modulating preeclamptic stress. Bratisl Lek Listy 2013;114:508-13.
92. Lindsay KL, Kennelly M, Culliton M, Smith T, Maguire OC, Shanahan F, *et al*. Probiotics in obese pregnancy do not reduce maternal fasting glucose: A double-blind, placebo-controlled, randomized trial (Probiotics in Pregnancy Study). Am J Clin Nutr 2014;99:1432-439.
93. Parrish MR, Martin JN Jr, Lamarca BB, Ellis B, Parrish SA, Owens MY, *et al*. Randomized, placebo controlled, double blind trial evaluating early pregnancy phytonutrient supplementation in the prevention of preeclampsia. J Perinatol 2013;33:593-9.

Source of Support: Nil, Conflict of Interest: None declared.