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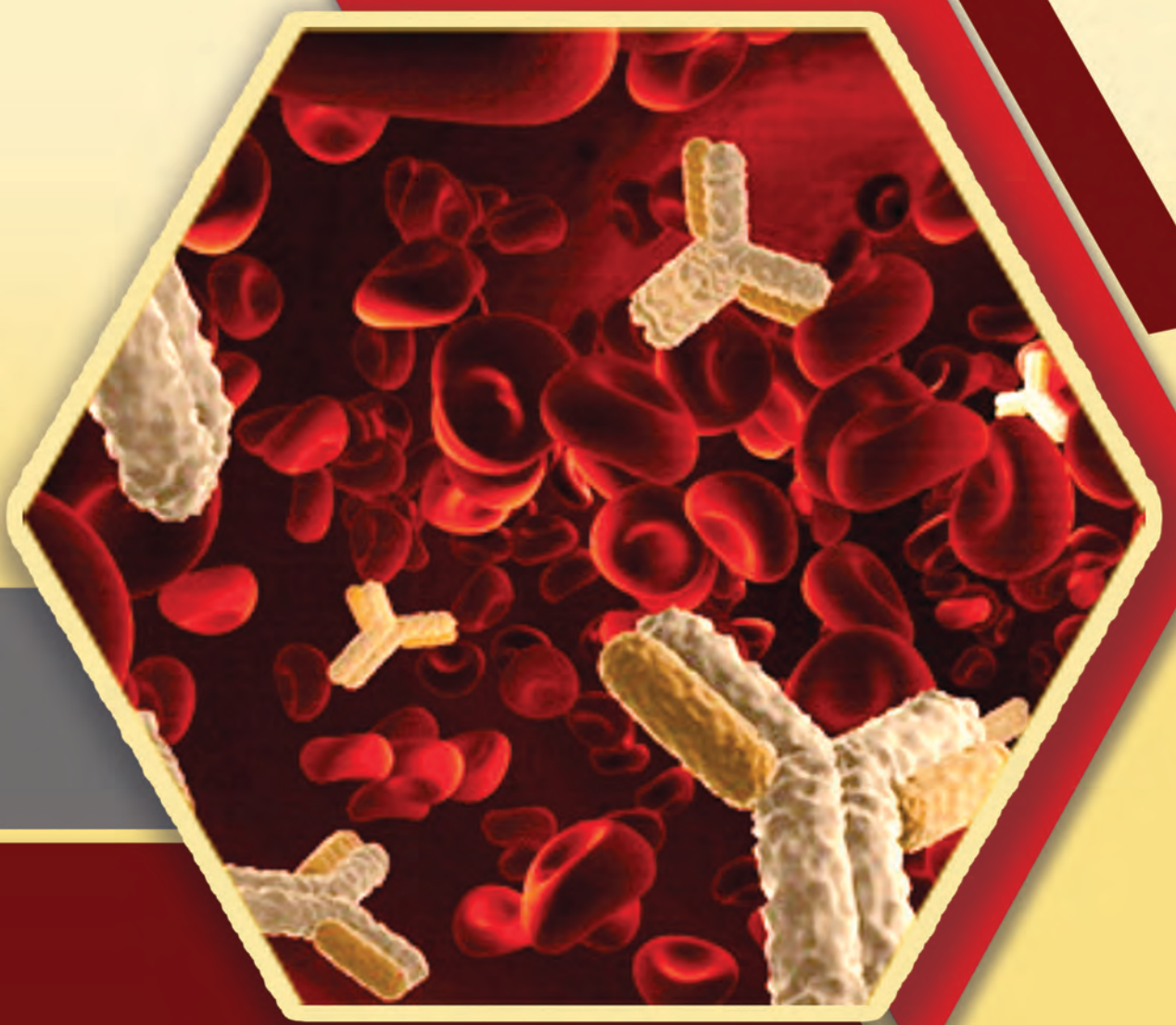

ICMR - NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY

Training program on “Basics of Flowcytometry” was organized by National Institute of Immunohaematology on 14-15th June, 2017. Project staff (15) under Centre of Excellence participated in workshop.

PIDCON 2017, CME on Pediatric Immune Deficiency was jointly organized by Indian Society of Pediatric Immune Deficiency, National Institute of Immunohaematology, Indian Association of Pediatrics and Department of Pediatrics, Government Medical College, Kozhikode on 9th July 2017. A total of 66 participants from all over India attended the CME.

Early prediction of preeclampsia using combination of plasma microparticles and serum biomarkers

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Summary:

Known as 'disease of theories', preeclampsia (PE) is a multisystem, heterogeneous disorder with confounding pathophysiology, characterized by sudden onset of hypertension and/ or proteinuria with other end organ dysfunction after 20 weeks of gestation in previously normotensive women [ACOG Guidelines, 2013]. It is the leading cause of indicated premature delivery and maternal death; affects up to 2-5% of pregnancies and is responsible for over 50000 maternal deaths annually worldwide [Khan et al, 2006]. PE not only affects the immediate outcomes of pregnancy at the time of delivery, but also the long-term cardiovascular health of the affected women and children. Women with history of PE have two to eight fold increased risk of myocardial infarction, stroke or diabetes mellitus [Mandruzzato et al, 2008]. The children born after pregnancies complicated with PE show increased risk of cardiovascular disease, hypertension, diabetes mellitus or renal disease during adult life.

Even though PE generally manifests in the late pregnancy, its underlying pathology largely takes place in the first trimester. This finding and absence of effective management has sparked great interest in the search for biomarkers for identification of PE before onset of their clinical manifestations. Early identification of women who will eventually develop PE will help in risk stratification and better therapeutic management.

Introduction:

Normal placental development is one of the important aspects of pregnancy which is associated with extensive vascular remodelling at maternal/fetal interface [Khong & Brosens, 2011]. The process of vascular remodelling ensures an adequate supply of blood to placenta and hence the developing fetus. The uteroplacental arteries undergo pregnancy-specific changes that include 1) apparent replacement of endothelium and media smooth muscle cells by invasive trophoblast, 2) loss of elasticity, 3) dilation to wide, incontractile tubes, and 4) loss of vasomotor control. The Extravillous trophoblast (EVT) replace the endothelial and smooth muscle cells of uterine spiral arteries and transform the spiral arteries from high-resistance and low-flow vessels into large-capacitance, low-resistance vessels [Burton et al, 2009]. The process of pseudovasculogenesis or endovascular transformation, begins in the late first trimester, ends by 18 to 20 weeks of gestation, in which the expression of a number of different classes of molecules such as integrins, cadherins, and metalloproteinases is modified as invading trophoblasts convert from an epithelial to an endothelial phenotype [Damsky & Fisher, 1998]. The spiral artery remodelling mediated

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by trophoblast invasion is an integral part of healthy pregnancy. Impaired spiral artery remodelling due to inadequate EVT invasion and incomplete endovascular transformation results in reduced blood flow in the maternal-fetal and placental interface in the first trimester of gestation. This has been implicated in pathophysiology of pregnancy disorders, such as PE, Intrauterine growth restriction (IUGR), gestational diabetes and small for gestation age (SGA) [Enquobahrie et al, 2008].

Preeclampsia:

PE is a pregnancy related condition identified by sudden onset of hypertension and either proteinuria or end-organ dysfunction after 20th week of gestation, can progress to convulsive state known as eclampsia if left untreated. The disease is complicated by cerebral hemorrhage, renal failure, epilepsy, stroke, respiratory insufficiency and kidney damage [Khan et al, 2006]. High incidence of PE in nulliparous women reflects inexperienced maternal immune response to pregnancy. The conventional risk factors for development of PE include chronic hypertension, diabetes, maternal age, obesity, previous PE, thrombophilia, autoimmune disorder, vascular disease, ethnicity and multiple births [Sargent et al, 2006].

The pathogenesis of PE remains poorly understood due to its heterogeneous, multi-systemic nature. Various theories have been put forward to explain the pathogenesis of PE which involves genetic predisposition, immune system dysregulation, placental ischemia, inflammation and so on. One of the most promising explanations of pathogenesis of PE is aberrant placental development as clinical manifestations of PE subside after removal of placenta [Roberts & Cooper, 2001]. The first stage is poor placentation which shows tight link between inadequate vascular remodelling; reduced blood flow in spiral artery before the 20th week of gestation and an increased risk of

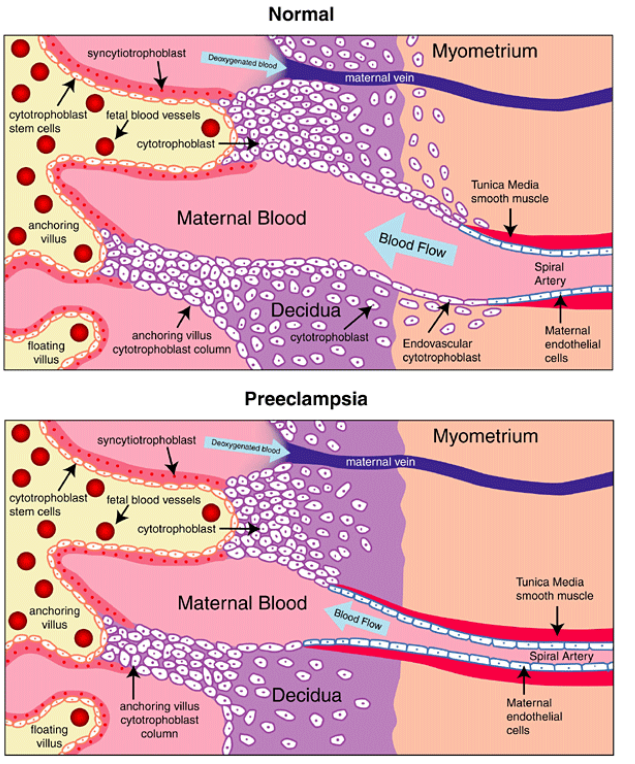

Figure 1: Abnormal Placentation in Preeclampsia [Adapted from Lam et al, 2005]

PE development [Hershkovitz et al, 2005]. The impaired spiral artery remodelling impedes an adequate response to increased blood flow demands of developing fetus. This leads to hypoxia/ placental ischemia and induces secretion of antiangiogenic, inflammatory factors such as cytokines, soluble fms-like tyrosine kinase 1 (sFlt1), antibodies of angiotensin-I receptors into maternal circulation [Maynard et al, 2003; Levine et al, 2004]. These changes result in second stage of maternal complications.

The maternal response to abnormal placentation represents the second stage of PE and characterized by systemic endothelial dysfunction [Roberts et al, 1989]. Various circulating factors such as sFlt1, soluble endoglin, interleukin 1, fibronectin, factor VIII antigen and tumor necrosis factor-α [Maynard et al, 2003; Levine et al, 2004; Venkatesha et al, 2006], etc. have been found elevated in pregnant women with PE. Concurrently, in PE, levels of endothelium-derived vasodilators such as nitric oxide (NO) [VanWijk et al, 2000] and

Biomarkers			Plasma/Serum Concentration compared to controls		
			1st Trimester	2nd Trimester	PE cases
Conventional	Angiogenic	Free VEGF	---	---	Reduced
		PLGF	Reduced	Reduced	Reduced
	Anti-angiogenic	sEng	---	Elevated	Elevated
		sFlt-1	---	Elevated	Elevated
	Immunological	PAPP-A	Reduced	Reduced	Reduced
		PP-13	Reduced	Elevated	Elevated
		P-Selectin	Elevated	Elevated	Elevated
	Endocrine	Activin A	Elevated	Elevated	Elevated
		Inhibin A	Elevated	Elevated	Elevated
		25-Hydroxy vitamin D	Reduced	Reduced	Reduced
Novel	Endocrine	Corin	--	--	Reduced
		Copeptin	Elevated	Elevated	Elevated
	Membrane vesicles	Microparticle*	---	---	Reduced/ elevated
	Circulating Nucleic Acid	miRNA**	Reduced/ elevated	Reduced/ elevated	Reduced/ elevated
	Proteins	Misfolded Protein#	---	Elevated	Elevated

Table 1: Biomarkers for prediction of preeclampsia [Adapted from Jadli et al, 2015]

* levels of microparticles were elevated or reduced depending on cell of origin.

** reduced or elevated expression of miRNA was according to miRNA subtype

\# Misfolded proteins were detected in urine congophilia test

prostacyclin are decreased in maternal serum and placenta. The imbalance between these beneficial and harmful circulating factors further triggers systemic inflammatory response along with endothelial dysfunction in organs such as kidney, liver, brain, vasculature etc. This multi-organ dysfunction eventually results in the development of the clinical symptoms of PE i.e. rapid onset of hypertension, proteinuria, and edema after 20th week of gestation [Redman & Sargent, 2005].

Biomarkers for prediction of PE

In the absence of effective preventive methods and heterogeneous presentation of PE, delivery remains only method of treatment. The symptoms of PE generally manifest in the second to third trimester of pregnancy but their underlying development largely takes place in the first trimester. Thus numerous studies investigated and proposed biomarkers for prediction of PE early in pregnancy before development of clinical symptoms. Early prediction will be beneficial for maternal and fetal surveillance, prophylactic use of anti-hypertensive drug, classification of patients into risk group etc. of these complications. Serum and plasma based biomarkers have been introduced along with biophysical methods such as Doppler for prediction of PE and IUGR.

CONVENTIONAL BIOMARKERS

Vascular Endothelial Growth Factor (VEGF)

VEGF is a proangiogenic factor that involved in proliferation and survival of endothelial cells. VEGF promotes vasculogenesis, angiogenesis and vascular permeability. VEGF exerts its biological effects by binding to tyrosine kinase receptors i.e. Flt-1 (VEGFR-1) and KDR (VEGFR-2, murine Flk-1) receptors present on vascular endothelial cells. Even though total VEGF has

been shown to be elevated in PE [Tsatsaris V et al, 2003], VEGF is bound by sFlt-1 in PE. This leads to low circulating levels of free VEGF in active PE. Due to higher affinity of VEGF to sFlt-1 compared to PlGF, the free VEGF concentration is significantly reduced in serum of pregnant women. One study reported VEGF as promising predictive marker for early onset preeclampsia but due to extremely low circulating concentrations of free VEGF (<30 pg/mL) and low detection limit of currently available ELISA kits, most of the studies found undetectable levels of VEGF [Taylor RN et al, 2003].

Placental Growth Factor (PlGF)

Placental growth factor (PlGF) is a pro-angiogenic factor of the VEGF family which is secreted by placental trophoblast. It plays a role in endothelial cell growth and control of vasculogenesis, angiogenesis, and placental development [Maynard et al, 2003]. Several studies reported low levels of PlGF in women who later developed PE compared to normotensive controls in early second trimester and as early as 10 to 11 weeks of gestation [Taylor et al, 2003; Levine et al, 2004]. Current evidence suggests that early and mid-pregnancy low levels of serum PlGF can distinguish women who subsequently develop preeclampsia from those who deliver an SGA infant without PE [Taylor et al, 2003].

Soluble Endoglin (sEng)

Endoglin (Eng) or CD105, is an angiogenic receptor, expressed on the surface of endothelial cells and placental syncytiotrophoblasts, acts as a co-receptor for transforming growth factor (TGF)-ß1 and TGF-ß3. It is involved in angiogenesis, regulation of the vascular tone and functions as a modulator of TGF-ß signalling [Cheifetz et al, 1992]. sEng is a circulatory form of endoglin consisting extracellular part of molecule due to proteocleavage of membrane bound endoglin [Venkatesha et al, 2006]. Compared to normotensive controls, soluble sEng is present in substantial excess in preeclamptic patients. The increase in concentration of no s-Eng is associated with severity of PE symptoms and concentration is highest in PE complicated by HELLP (Hemolysis, Elevated Liver enzymes and Low Platelet count) syndrome [Venkatesha et al, 2006].

Soluble Fms-Like Tyrosine Kinase-1 (sFLT-1)

Soluble fms-like tyrosine kinase-1 (sFlt-1) is a secreted protein, a splice variant of the VEGF receptor Flt-1, which lacks the transmembrane and cytoplasmic domains of Flt-1. Circulating in blood, it acts as potent antagonist of VEGF and PlGF by preventing their interaction with their endogenous receptor. Secreted in large amounts by PE placenta, sFlt-1 exerts its antagonistic effect by binding to VEGF and PlGF which leads to decreased levels of VEGF and PlGF in women with PE. Circulating sFlt-1 levels are increased in women with established PE and may begin to rise before the onset of clinical symptoms i.e. proteinuria, Hypertension, and glomerular endotheliosis [Levine et al, 2006].

Pregnancy-associated plasma protein A (PAPP-A)

PAPP-A is a disulfide bond linked homodimeric peptidase of 1628 amino acids mainly produced by the placental trophoblasts [Grill et al, 2009]. It is responsible for the cleavage of insulin-like growth factor binding proteins (IGFBP-2 and IGFBP-4), that play an important role in regulating fetal growth. Reduced levels of PAPP-A may result in increased amounts of insulin-like growth factor (IGF) being bound to its carrier proteins and hence not available at the cell receptor level to stimulate fetal growth and trophoblast invasion of the decidua. The maternal plasma concentration increases throughout pregnancy. In recent years decreased plasma levels of PAPP-A have been reported in pregnancy complicated by PE [Spencer et al, 2007].

Placental Protein 13 (PP13)

Placental protein 13 (PP13) is a 32 kDa dimeric protein, member of galectin family and is produced by placental trophoblast cells [Than et al, 2004]. The function(s) of PP13 is still not clearly understood, but it is involved in placenta implantation and maternal vascular remodelling [Nicolaides et al, 2006; Grill et al, 2009]. Serum levels of PP13 increases slowly with gestational age but reduced levels were reported in first trimester serum samples of women subsequently developing fetal growth restriction and PE, in particular cases with early onset [Nicolaides et al, 2006; Spencer K et al, 2007]. During Second and third trimester, levels of PP13 were found to be high in all risk groups which included PE, IUGR and preterm delivery, indicating utility of PP13 as an early predictor marker of risk of PE at first trimester [Grill et al, 2009].

P-Selectin

P-selectin is a member of the selectin family of cell surface adhesion molecules. It is expressed in α granules of platelet and the Weibel-Palade bodies of endothelial cells and is involved in leucocyte-endothelial interactions [Larsen et al, 1989]. P-selectin is rapidly shed from the cellular membrane of activated platelets and this release is suggested to contribute to most of the soluble isoform of the molecule that is found in the plasma. As preeclampsia is associated with extensive platelet activation, platelet derived microparticles with P-selectin have been detected in the peripheral blood of preeclamptic women [Bretelle et al, 2003; Lok et al, 2008]. Elevated levels of soluble P-Selectin in serum or plasma have been reported in some studies involving women with PE [Chaiworapongsa et al, 2002].

25-Hydroxyvitamin D [25-(OH)D]

Vitamin D is a seco-steroid pro-hormone which is transported to the liver from the skin and intestines by vitamin D-binding protein (DBP). For biological activation, it undergoes two successive hydroxylations, firstly to 25-hydroxy vitamin D (25(OH)D) and then to 1,25-dihydroxyvitamin D (1,25(OH)2 D), i.e. calcitriol. 25- hydroxyvitamin D (25(OH)D) is major form of vitamin D found in blood and a nutritional biomarker for vitamin D status [Dusso, 2011]. Robinson et al suggested association between low maternal 25-hydroxyvitamin D (25(OH)D) and risk of early onset PE [Robinson et al, 2013]. A prospective cohort study estimated vitamin D status in pregnant women at 12–18 and 24–26 weeks of gestation. 39% women of cohort found to be vitamin D deficient (25(OH)D <50 nmol/l). Maternal plasma vitamin D levels were significantly low in women who developed PE compared to women with normal pregnancy outcome [Wei et al, 2012].

NOVEL BIOMARKERS

Corin

Atrial natriuretic peptide (ANP) is a cardiac hormone which plays an important role in regulation of blood pressure and sodium homeostasis [Wu et al, 2009]. For physiological effect of ANP, it must be converted from inactive precursor ANP (pro-ANP) to ANP. Identified in 1999 as a novel serine protease in heart, corin is a 1042 amino acid transmembrane protein which activates pro-ANP to active ANP. [Wu et al, 2009].

In nested case control study, drawn from prospective longitudinal study of 122 women with singleton pregnancy, including 12 preterm-PE and 13 term-PE, maternal plasma levels of corin and mid-regional proatrial natriuretic peptide (MR-PANP) were analyzed before 20 weeks of gestation. The $\log_{10}$ corin and $\log_{10}$ MR-PANP levels were correlated with gestational age. While $\log_{10}$ corin levels were significantly associated with gestational age ($p<0.01$); $\log_{10}$ MR-PANP levels were not significant. Corin and MR-PANP levels were significantly altered in preterm-PE cases compared to normotensive controls ($p=0.001$ and $p=0.046$ respectively). Both the markers did not show significant change for term-PE cases [Khalil et al, 2015].

The study showed significantly reduced levels of corin in women destined to develop PE.

Copeptin (CPP)

Arginine vasopressin (AVP), known as anti-diuretic hormone, is one of the main hormones of hypothalamic-pituitary-adrenal axis. It acts as a regulator of homeostasis of cardiovascular and renal system. Multiple studies elucidated possible relationship between vasopressin and pregnancy and hence its role in pathophysiology of PE. Short biological half-life (5-20 minutes) in blood, small size and most of it found in platelet bound form (more than 90%) in circulation complicate measurement of AVP by known analytical methods. These limitations impair use of AVP as a biomarker in clinical settings.Copeptin is a 39-amino acid C-terminal component of prepro-vasopressin. Copeptin is produced in 1:1 stoichiometric ratio with AVP and relatively stable in serum and plasma [Meyer et al, 2009]. In a study, Santillan et al showed that the copeptin levels in plasma at 6 weeks of gestation in pregnant women who subsequently developed PE were significantly higher than women who remained normotensive during pregnancy [Santillan et al, 2014]. In another prospective cohort study, maternal serum copeptin levels at mean 16 weeks gestation found to be significantly higher in severe PE cases and showed strong association with risk of preterm-PE after adjustment of covariates in multiple regression analysis. This study demonstrated not only elevation of copeptin levels with gestational age in both PE and control groups but also specific association with PE and not with other pregnancy associated complications [Yeung et al, 2014].

Microparticles

Described first by Wolf in 1967 as "dust" procoagulant formation around an activated platelet [Wolf, 1967], Microparticles (MPs) are now known as heterogeneous population of fragments (0.05-1.0 µm), released from the cell membranes of variety of cells such as platelets, granulocytes, erythrocytes, endothelial cells during apoptosis or activation. MPs play a key role in cell signalling and cell to cell communication by transfer of miRNA and mRNA from parent cells to target cells. MPs exert physiological effects such as cell differentiation, angiogenesis and inflammation.

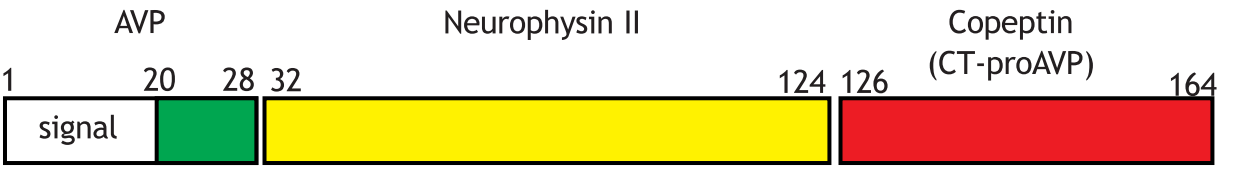

Figure 2: Structure of Pre-pro-vasopressin

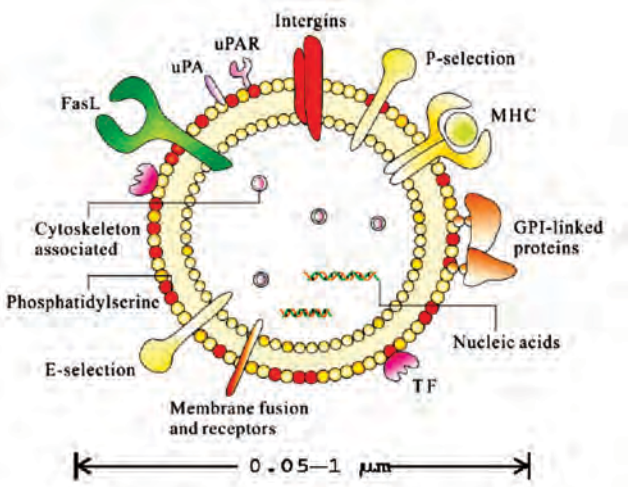

Figure 3: Structure of Microparticles [Adapted from Tan et al, 2014]

In a longitudinal study assessing change in plasma levels of various cell derived MPs, Lok et al. reported significantly reduced number of total MPs (p=0.04) in patients with PE at 28-36 weeks of gestation. While erythrocyte derived MPs were elevated at 28 weeks (p = 0.04), monocyte derived MPs were elevated at 28 (p= 0.007), 32 (p=0.02) and 36 (p=0.01) weeks of gestation in women with PE. Significantly low levels of PMPs were observed in PE patients (p = 0.03) at 28 weeks of gestation [Lok et al, 2008]. Bretelle et al reported low levels of PMPs in complicated pregnancies, but significantly low levels in women who developed PE. Consumption of PMPs in placental beds of pregnancy complicated by PE, by excessive clotting reactions or binding to fibrin deposits is implicated in reduction of PMP levels [Bretelle et al, 2003]. Very few studies have tried to predict PE before onset of clinical manifestations using MPs as biomarkers, but most of the studies are on comparative estimation of circulating MPs (cMPS) of different cell origins in PE cases and healthy normal pregnant women.

NIIH Experience:

In a nested case-control study derived from a prospective cohort comprising 772 primigravid women were included for investigation of role of cMPs and serum biomarkers, alone and in combination, for prediction of PE at 10-14 weeks of gestation. 33 women (4.76%) who subsequently developed PE, 81 (11.68%) who developed IUGR and 112 women who matched with the gestational age at blood collection and storage time with normal delivery outcome were used as controls. PE cases were further classified, depending on clinical presentation as isolated PE (16/33) and PE complicated by IUGR (17/33).

Methodology for analysis of MPs has been standardized on Becton Dickinson (BD) fluorescence activated cell sorting (FACS) Aria. Measurement of serum concentrations of PlGF, PAPP-A, PP-13, 25-hydroxyvitamin D, sEng, sFlt-1, P-selectin were done using commercial ELISA kits. The plasma cMPs and serum biomarker concentrations were expressed as median (interquartile range). Comparison of clinical characteristics and biomarker levels between PE, IUGR and control groups were done using Mann-Whitney test. Statistical significance was assumed at $P < 0.05$. Sensitivity and specificity for individual marker was calculated and depicted as Receiver operating characteristic (ROC) curves. Binary logistic regression analysis was used to evaluate predictive utility of combination of biomarkers for PE and IUGR prediction.

Women who subsequently developed PE showed significantly elevated levels of total annexin V MPs (p<0.0001), CD41a (p-0.0001), CD62e (p-0.006), CD146 (p-0.0143), CD45 (p-0.0005), CD235a (p-0.0005) along with serum PP-13 (p-0.0414), P-Selectin (p<0.0001), sFlt-1 (p-0.001) and CPP (p<0.0001) compared to gestational age matched controls at 10-14 weeks of gestation. Serum PlGF levels were significantly reduced in women with PE (p<0.0001) compared to controls. No statistically significant alteration was observed in biomarker levels in women who developed IUGR as compared to controls. The combination of total annexin V MPs, PlGF and CPP showed Area under curve (AUC), sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV), Positive likelihood ratio (PLR), and Negative likelihood ratio (NLR) of 0.970 (0.946-0.995), 93.1%, 91.8%, 77.50%, 98.10%, 11.69 and 0.07 respectively for prediction of PE at 10-14 weeks of gestation. The combination of total annexin V MPs, PlGF and CPP showed poor predictive value for prediction of IUGR. These markers showed good predictive values, alone and in combination, for prediction of subgroups of PE i.e. isolated PE and PE cases complicated by IUGR. While combination showed 0.966 (0.934-0.999) AUC with 91.7% sensitivity, 92.8% specificity, 65.22% PPV, 99.05% NPV, 13.13 PLR, and 0.07 NLR for 10-14 weeks prediction of isolated PE cases, with AUC of 0.975 (0.947-1.000), 100% specificity, 90.7% specificity, 60.71% PPV, 100% NPV, 10.18 PLR, and 0.00 NLR, the combination showed high

predictive utility for prediction of PE cases complicated by IUGR. This is the first nested case control study which showed promising results for prediction of PE using circulating MPs in combination with serum PlGF and CPP. This appears to be an ideal combination for PE prediction at 10-14 weeks of gestational age and will also be useful in discriminating patients at risk of developing PE from other pregnancy complications early in gestational age.

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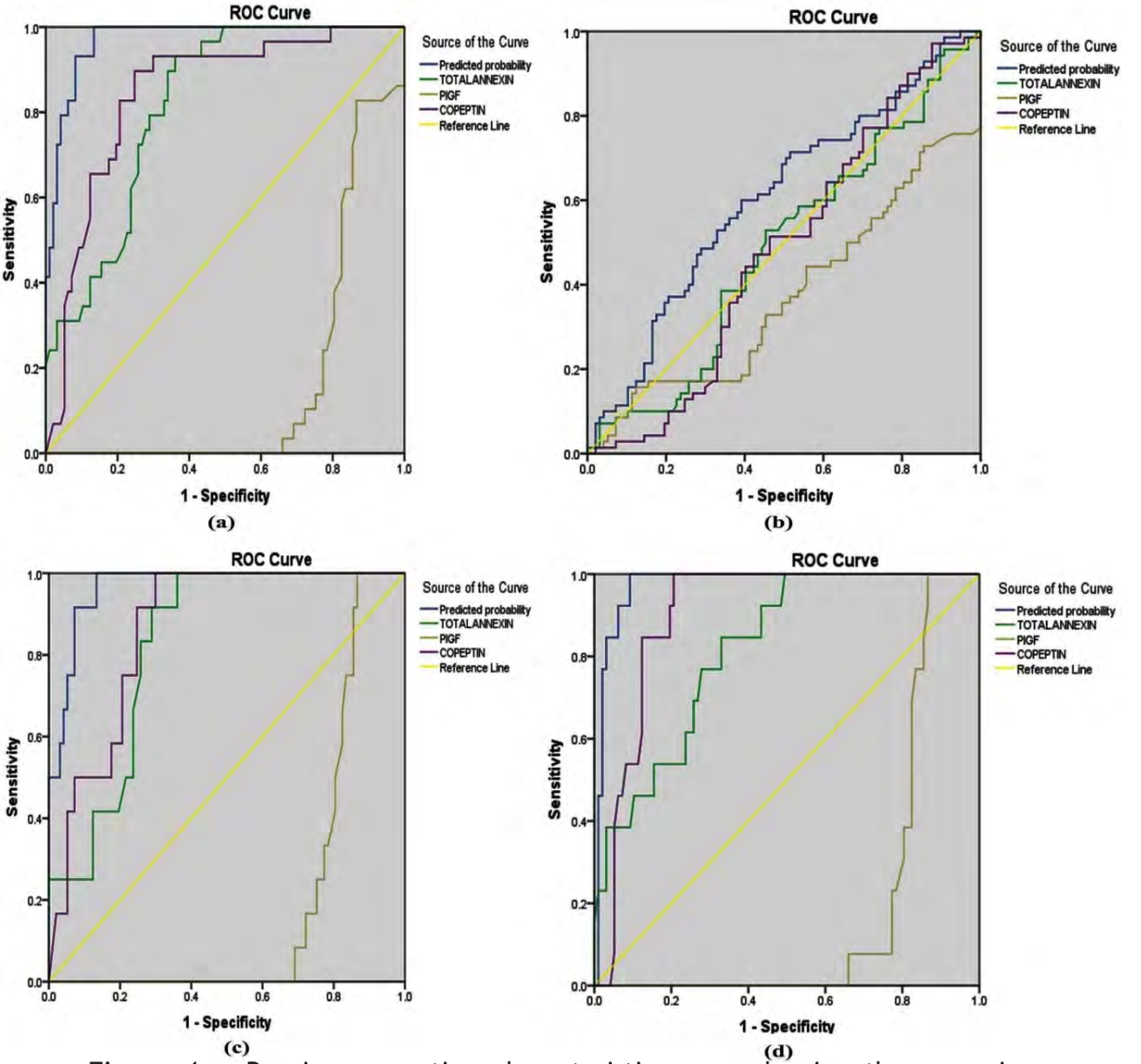

Figure 4: Receiver operating characteristic curve showing the screening characteristics of Total annexin V MPs, PlGF and CPP, alone and in combination for prediction of (a) PE (b) IUGR (c) Isolated PE (d) PE complicated by IUGR.

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सारांश

प्लाज्मा सूक्ष्मकण (Microparticles) और सीरम बायोमार्कर की मदद से प्राक्गभक्षिपेक (P.E) की शीघ्र पूर्वसूचना

मनिषा पटवर्धन

नाल विकास (Placental development) गर्भावस्था की बहूत महत्वपूर्ण घटना है । गर्भ को पर्याप्त खून पहुँचाने का कार्य इसी द्वारा होता है। गर्भावस्था के नौ महिनो में नाल प्रणाली की अवस्थाओं में निरंतर बदलाव आते हैं। नाल प्रणाली में किसी भी प्रकार की गड़बड़ी गर्भ के विकास में बाधा डालती हैं। गर्भ में मस्तिष्क का अविकसित रहना, ऐंठन (Convulsion) की बिमारी पैदा होने का अंदेशा नाल विकास पर निर्भर रहता हैं ।

प्राक्गभाक्षिपेक (P.E) गर्भावस्था में निर्माण होनेवाली गंभीर बिमारी हैं । इस विकार में गर्भवती स्त्री के शरीर में गर्भ के २० वे सप्ताह में उच्च रक्तचाप एवं मूत्र में बढती मात्रा में प्रोटीन आदि लक्षण दिखाई देने लगते हैं । P.E का गंभीर रुप,समयपूर्व गर्भपात और माताओं के मृत्यू का कारण बन सकता हैं। विश्व में लगभग 50,000 गर्भवती महिलाओं की मृत्यू P.E से होती हैं । 2 से 5% गर्भधारणाओंमे P.E से गर्भपाात होते हैं। P.E, माता और शिशूओं में हृदयसंबंधी बिमारीयों का खतरा बढाती हैं। P.E बाधित माताओं के बच्चों को बढती उम्र में उच्च रक्तचाप, मधुमेह, हृदयरोग जैसी बिमारियां हो सकती हैं । P.E आसानी से पहचान में नहीं आती अत: उसका प्रबंधन दुष्कर हैं । बायोमार्कर की मदद से शुरुआती अवस्था में P.E को पहचाना जा सकता है ।

P.E की पूर्वसूचना देनेवाले बायोमार्कर :

P.E की पूर्वसूचना आसानी से संभव नहीं। किंतु कई बायोमार्करों समय समय पर निरीक्षण करने से प्रारंभ मे ही P.E की पूर्वसूचना मिल सकती हैं। निम्नलिखित बायोमार्कर का अध्ययन दुनियाभर के वैज्ञानिक कर रहे हैं।

१) **नाल विकास घटक** (Placental growth factor (PlGF)

गर्भावस्था में शुरुआती और मध्य गर्भावस्था मे अगर इस घटक मात्रा में कमी पाई जाती है, तो मरीज को P.E का खतरा लाहक है ।

२) **टायरोसिन कायनेज १** (sFLT-1)

घुलनशील P.E के मरीज में बढते समय के साथ इस प्रोटीन की खुन में रसी मात्रा भी बढ़ जाती है । इसी के साथ मरीज में रक्तचाप, मुत्र में प्रोटीन का बढना आदि लक्षण साँज्ञा होने लगते है ।

३) **घुलनशील एन्डोग्लीन** (sEng)

साधारण जनसंख्या के मुकाबले P.E के मरीजों के सीरम में एन्डोग्लीन की मात्रा काफी ज्यादा पायी जाती है । P.E के तीव्र लक्षण बढ़ी हुई एन्डोग्लीन से मेल खाती है ।

४) **गर्भवस्था संबंधी प्लाज्मा प्रोटीन 'ए'** (PAPP-A)

आम तौर पर गर्भावस्था की बढ़ती अवस्था में PAPP-A की मात्रा बढ़ती है । P.E के जटिल होने पर प्लाज्मा PAPP-A का स्तर गिरने लगता है ।

५) **प्लाज्मा प्रोटीन** 13 (PP-13)

गर्भावस्था के शुरुआत के तीन माह में जहाँ साधारण गर्भावस्था का PP-13 स्तर बढता है, वहाँ P.E ग्रस्त मरीज में इस में गिरावट पायी जाती है । हालांकि दुसरी और तिसरी तिमाही मे PP-13 लगातार बढ़ती है! इस कारण गर्भावस्था की पहली तिमाही में P.E का पता लगाने के लिए PP-13 महत्वपूर्ण बायोमार्कर के रुप मे उभरी है ।

६) **पी - सिलेक्टीन** (P-selectin)

खून का असाधारण रिसाव रोकने का काम बिंबाणू (Platelets) कोशिका करती हैं । P.E की बिमारी में बिंबाणू कोशिका का उत्तेजन एक आम बात हैं । ऐसे में प्लाज्मा और सीरम में P-selectin की लगातार बढ़ती मात्रा P.E का संकेत देती है।

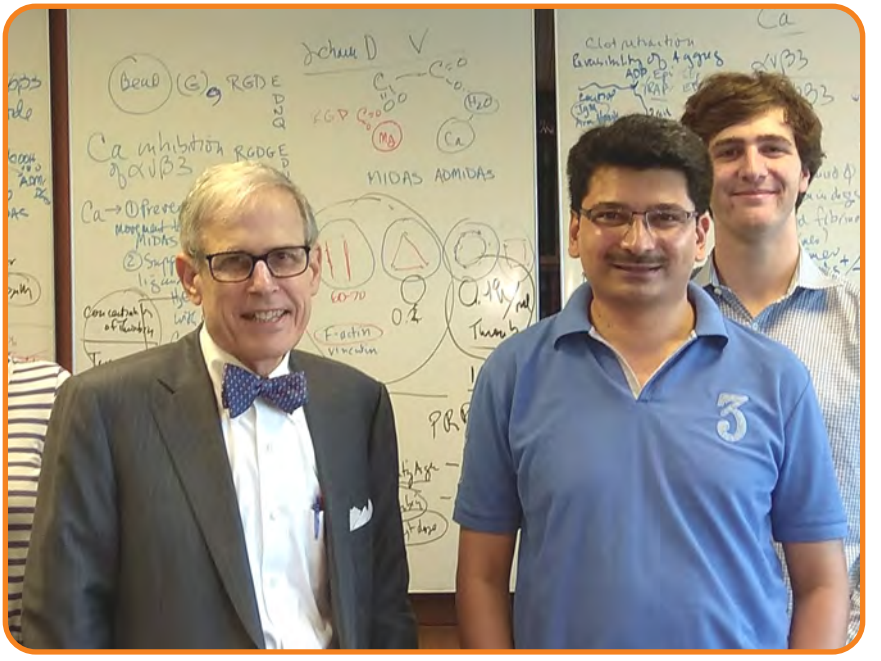
७) **हायड्रोक्सिविटामिन डी** [25(OH)D]

गर्भवती स्त्री में लगातार कम हायड्रोक्सिविटामिन डी, P.E की और इशारा करते हैं । हालांकि विटामिन डी की कमी कई विकारो को दर्शाती है ।

८) **कोपेप्टीन** (Copeptin)

पियूषिका (Pituitary) ग्रंथी से आर्जिनीन वॅसोप्रेसिन (AVP) नामक हार्मोन रिसता हैं! इस की पूर्व अवस्था 'कोपेप्टीन' से जानी जाती है । P.E से प्रभावित गर्भावस्था में (16 हप्तों मे) सीरम कोपेप्टीन का उच्च मात्रा में पाना P.E की ओर इशारा करता है । बायोमार्कर के तौर पर कोपेप्टीन की पहचान हुई है । P.E का विशिष्ट (Specific) (बिंबाणू से निकलनेवाले) सूक्ष्मकण (Platelet microparticles) शरीर में कई प्रकार की कोशिका सूक्ष्मकणो को सीरम में छोडती है । बिंबाणू (Platelet) से निकलनेवाले रक्तकण खास तौर से खून का थक्का (Clot) बनने मे मदद देते है । गर्भावस्था मे स्त्री के खून में इन सूक्ष्मकणो को जाँचने के लिए कई अध्ययन किये गये । जटील गर्भावस्था (Complicated Pregnancy) मे बिंबाणू-सूक्ष्मकण (PMP) में भारी कमी पायी गयी। हालांकी उनका संबंध P.E से सीधे तौर पर प्रस्थापित नही हो पाया ।

NIIH **मे** P.E का अध्ययन: पहिली बार गर्भधारण करनेवाली 772 स्त्रियाओं (10 से 14 हप्तों की गर्भावस्था में) को NIIH में किए गये अध्ययन मे शामिल किया गया था। इनमें कई बायोमार्कर जाँचे गए । अध्ययन गूट में से 33 गर्भवती महिलाओं मे PE, 81 मे गर्भ का विकास धीमा (Intrauterine Growth Retardation, IUGR) पाया गया । जिन गर्भवती महिलाओं मे P.E स्थापित हुई उनमे अनेक्सिन -5 (annexin V), पी सिलेक्टिन नियंत्रित गुट से (Control group) काफी ज्यादा मात्रा मे पाए गये। किंतु नाल विकास घटक (PIGF) घटा हुआ मिला । जिन गर्भवती महिलाओं में महज गर्भ के धीमे विकास की शिकायत थी, उनमें उपरोक्त बायोमार्कर का अनुमात P.E के मुकाबले सामान्य था । इस अध्ययन से अनुमान लगाया जा सकता है की सीरम नाल विकास घटक (SPIGF) और सूक्ष्मकणों की तादाद के अनुपात से P.E की पूर्वसूचना मिलना संभव है।



Dr. Bipin P. Kulkarni, Scientist C received ICMR- International Fellowship award for Young Biomedical Scientists, for the year 2016- 2017. He visited Dr. Barry Coller's laboratory at the Rockefeller University, New York Genome Centre and Mount Sinai Centre for Genomics, New York, from 24th February to 22nd August 2017. He was trained to use various NGS platforms, wet bench processes for genomic and exomic library preparations, clustering of the libraries and sequencing runs on Illumina HiSeq 2500 sequencer and processes involving bioinformatic analysis of the sequencing data. This training will be utilized for setting up NGS facilities for inherited disorders at NIIH.

Annual training programme in Immunohaematology: The training programme is an orientation course for medical officers and laboratory technicians currently working in blood banks. The training programme was held from 10th March to 9th April, 2017. Seventeen medical officers and two technicians and from all over India attended the training programme.

ANNUAL TRAINING PROGRAMME IN IMMUNOHAEMATOLOGY

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