Hindawi
Journal of Pregnancy
Volume 2018, Article ID 2632637, 23 pages
https://doi.org/10.1155/2018/2632637

Review Article

Clinical Presentation of Preeclampsia and the Diagnostic Value of Proteins and Their Methylation Products as Biomarkers in Pregnant Women with Preeclampsia and Their Newborns

Maria Portelli and Byron Baron

Centre for Molecular Medicine and Biobanking, Faculty of Medicine and Surgery, University of Malta, Msida MSD2080, Malta

Correspondence should be addressed to Byron Baron; angenlabs@gmail.com

Received 4 February 2018; Accepted 15 May 2018; Published 28 June 2018

Academic Editor: Fabio Facchinetti

Preeclampsia (PE) is a disorder which affects 1-10% of pregnant women worldwide. It is characterised by hypertension and proteinuria in the later stages of gestation and can lead to maternal and perinatal morbidity and mortality. Other than the delivery of the foetus and the removal of the placenta, to date there are no therapeutic approaches to treat or prevent PE. It is thus only possible to reduce PE-related mortality through early detection, careful monitoring, and treatment of the symptoms. For these reasons the search for noninvasive, blood-borne, or urinary biochemical markers that could be used for the screening, presymptomatic diagnosis, and prediction of the development of PE is of great urgency. So far, a number of biomarkers have been proposed for predicting PE, based on pathophysiological observations, but these have mostly proven to be unreliable and inconsistent between different studies. The clinical presentation of PE and data gathered for the biochemical markers placental growth factor (PlGF), soluble Feline McDonough Sarcoma- (fms-) like tyrosine kinase-1 (sFlt-1), asymmetric dimethylarginine (ADMA), and methyllysine is being reviewed with the aim of providing both a clinical and biochemical understanding of how these biomarkers might assist in the diagnosis of PE or indicate its severity.

1. Introduction

Preeclampsia (PE) is a multisystem, pregnancy-specific disorder that is characterised by the development of hypertension and proteinuria (elevated levels of protein in the urine) after 20 weeks of gestation [1]. PE is a leading cause of maternal, perinatal (from the 20th week of gestation to the 4th week after birth), and foetal/neonatal mortality and morbidity worldwide [2, 3].

PE is a very significant disease which complicates from 2% to 5% of pregnancies in Europe and America and can reach up to 10% of pregnancies in developing countries, mainly due to the lack of or inadequacy of emergency care [2]. Also, PE is associated with an increased risk of placental abruption, preterm birth, foetal intrauterine growth restriction (IUGR), acute renal failure, cerebrovascular and cardiovascular complications, disseminated intravascular coagulation, and maternal death [4]. Therefore, the ability to provide an early diagnosis of PE is vital.

2. Clinical Presentation, Diagnosis, and Pathophysiology of PE

Clinically, PE presents as new-onset hypertension in a previously normotensive woman, with systolic and diastolic blood pressure readings of ≥140 and ≥90 mmHg, respectively, on 2 separate occasions that are at least 6 hours apart, together with proteinuria that develops after 20 weeks of gestation [5–7].

This disorder can have an early onset (PE starting before 34 weeks of gestation) or late onset (after 34 weeks of gestation) and can be classified as mild or severe, depending on the severity of the symptoms present [2] (Table 1). In the

Table 1: Symptoms presented by patients with mild and severe PE. The diagnosis of any form of PE requires the presentation of both hypertension and proteinuria. This may be accompanied by a multitude of other symptoms if the PE is severe [8, 9].

Symptom	Mild PE	Severe PE
Blood Pressure	Systolic ≥140 mm Hg or diastolic ≥90 mm Hg, over 20 weeks of gestation (in a woman with previously normal blood pressure)	Systolic ≥160 mm Hg or diastolic ≥110 mm Hg (on two occasions at least six hours apart; in a woman on bed rest)
Proteinuria	24-hour urine collection protein ≥0.3 g (urine dipstick test ≥1+)	24-hour urine collection protein ≥5 g (urine dipstick test ≥3+; in two random urine samples collected at least four hours apart)
Others	N.A.	(i) Oliguria (ii) Cerebral or visual disturbances (iii) Pulmonary oedema or cyanosis (iv) Epigastric or right upper quadrant pain (v) Impaired liver function (vi) Thrombocytopenia (vii) Intrauterine growth restriction

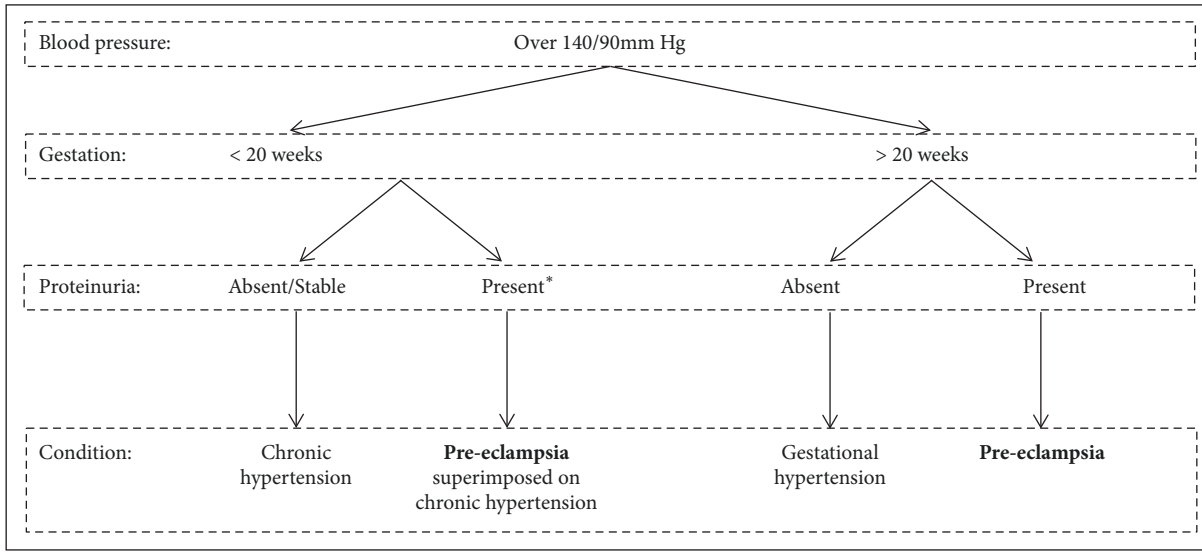

Figure 1: Simplified diagnostic information for the distinction of different types of hypertension and preeclampsia. ∗In the form of new or increased proteinuria, together with development of increasing blood pressure, or HELLP syndrome [9].

case of severe PE, more significant blood pressure elevations and a greater degree of proteinuria are noted. Other symptomatic features of severe PE which may be present include oliguria (less than 500 mL of urine in 24 hours), cerebral or visual disturbances, and pulmonary oedema or cyanosis [8, 9].

Also, the clinical presentation of PE may be either insidious or fulminant since some women may be asymptomatic initially, even after hypertension and proteinuria are noted, while others may present symptoms of severe PE from the start [1]. Finally, this condition may present itself as a maternal disorder only, such that there is normal foetal growth, or else it may lead to intrauterine growth restriction or sudden foetal distress [2].

Hypertensive disorders of pregnancy are the most common complications seen by obstetricians [10] and they are all associated with higher rates of maternal and foetal mortality and morbidity [11]. This category of disorders includes chronic hypertension, PE, PE superimposed on chronic hypertension, and gestational hypertension [9]. The aetiologies and pathology of these disorders vary, and thus obtaining a diagnosis of PE becomes less difficult if physicians are able to differentiate PE from the other hypertensive disorders of pregnancy (Figure 1).

In chronic hypertension, the elevated blood pressure may predate the pregnancy, be noted before 20 weeks of gestation, or else be present 12 weeks after delivery [9]. This contrasts with PE, which is defined by the presence of elevated blood pressure and proteinuria after 20 weeks of gestation. In severe cases, PE can evolve into eclampsia which is a severe complication that is characterised by new-onset of epileptic seizures (generalised convulsions), due to angiospasms in the brain and brain oedema [13], in a woman with PE [1]. Eclampsia usually occurs in the second half of pregnancy and

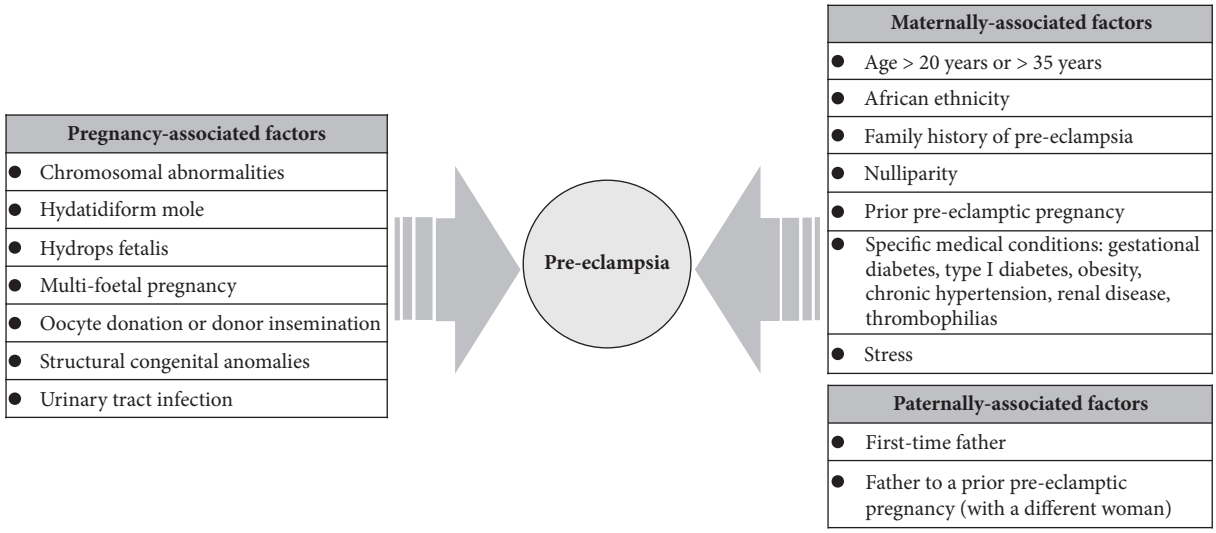

Figure 2: Risk factors for PE associated with the pregnancy itself or with specific parental characteristics from both maternal and paternal side [8, 12].

is a significant cause of maternal death, most commonly as a result of cerebral haemorrhage [14].

PE superimposed on chronic hypertension is characterised by new-onset proteinuria (or by a sudden increase in the protein level if proteinuria was already present), an acute increase in blood pressure (assuming proteinuria already exists), or the development of the HELLP (haemolysis, elevated liver enzyme, low platelet count) syndrome [8]. Finally, gestational hypertension can be distinguished from PE since it is characterised by the presence of elevated blood pressure after 20 weeks of gestation, which normalises within 12 weeks after delivery, together with the absence of proteinuria [8].

Medical conditions which have a potential to cause microvascular disease, including diabetes mellitus, chronic hypertension, and vascular and connective tissue disorders, as well as antiphospholipid syndrome and nephropathy, are all risk factors for developing PE. A number of other risk factors for developing PE, which can be associated with the pregnancy itself or with the clinical characteristics of the mother or father of the foetus, are presented in Figure 2 [8, 12, 15].

Although the pathophysiology of PE is not fully understood, problems of placental implantation and the level of trophoblastic invasion, as a consequence of endothelial dysfunction, appear to play a central role in the development and progression of this disorder. During normal pregnancy, cytotrophoblasts derived from the foetus invade and remodel the maternal uterine spiral arteries such that these small diameter, high-resistance arteries are converted into high capacity, low-resistance vessels [16]. This process is completed around midgestation in order to optimise the distribution of the maternal blood and ensure that the developing uteroplacental unit has adequate oxygen and nutrient delivery from the maternal circulation (Ramsey and Donner (1980) as cited in [3]).

PE is thought to evolve in two stages. The first, asymptomatic stage of PE involves impaired trophoblastic invasion of the decidua (maternal placental bed) that seems to be due to local, abnormal foetomaternal immune interactions within the uterine wall [2, 17, 18]. This abnormal, shallow placentation reduces uteroplacental blood perfusion and consequently leads to local placental hypoxia. This oxidative stress has been shown to further aggravate vascular function in the placenta [19], which consequently leads to insufficient blood perfusion, inflammation, apoptosis, and structural damage [17, 20–23].

In the second stage, placental blood-borne factors released into the maternal circulation from the poorly perfused placenta, together with the aberrant expression of proinflammatory, antiangiogenic, and angiogenic factors, may activate the maternal endothelium and will eventually cause the endothelial dysfunction that leads to the main clinical symptoms of PE: hypertension and proteinuria [14, 24]. It has been noted that the magnitude of defective trophoblastic invasion of the spiral arteries correlates with the severity of PE [25].

Although PE is not preventable, PE-related mortality can be decreased through early detection and careful monitoring of PE [1]. Also, women who have progressive or severe PE should be hospitalised early on to allow close monitoring of both the maternal and foetal health condition.

3. Biomarkers of PE

The search for noninvasive, blood-borne, or urinary biomarkers that could be used to screen for and diagnose this life-threatening disorder of pregnancy is of utmost importance. Such biomarkers could predict the development of PE or assist in its detection, which in turn could have a vital impact on the management of pregnant women and their unborn children [2].

Most significantly, screening pregnant women with the use of biochemical markers for PE could enable presymptomatic diagnosis which will in turn reduce unnecessary

suffering and healthcare costs associated with this disorder [26]. By providing an earlier diagnosis, progression of the disorder can be monitored more closely, together with the maternal and foetal health condition, thus allowing for more optimised time for delivery with the aim of reducing the number of premature births or other complications associated with PE [27]. Such biochemical markers may also allow the categorisation of women with PE according to the severity of the symptoms and/or pregnancy outcome which would further improve their clinical management [28].

Numerous biochemical markers for PE, which were selected based on pathophysiological observations noted in cases of PE, such as placental and/or endothelial dysfunction, have been investigated (Table 2). However, the reliability of these markers in predicting PE has been inconsistent between different studies [2]. Consequently, this review will focus on those biochemical markers for PE which appear to be most clinically relevant, alone or in combination, for the diagnosis of PE as well as in their ability to give an indication of the severity of this disorder, namely, placental growth factor (PlGF), soluble Feline McDonough Sarcoma- (fms-) like tyrosine kinase-1 (sFlt-1), and asymmetric dimethylarginine (ADMA), as well as introducing the possibility of screening for methyl-lysine in pregnancy-related proteins.

3.1. Placental Growth Factor (PlGF) and Soluble fms-Like Tyrosine Kinase-1 (sFlt-1). PlGF belongs to the vascular endothelial growth factor (VEGF) family of proteins and it shares 53% identity with the platelet-derived growth factor-like region of VEGF [29]. Based on this homology with VEGF, PlGF was proposed to be an angiogenic factor [29–31]. In fact, PlGF was seen to possess strong angiogenic and mitogenic properties which are capable of inducing the proliferation, migration, and activation of endothelial cells [32, 33].

The expression of PlGF messenger RNA (mRNA) appears to be restricted to the placenta, trophoblastic tumours, and cultured human endothelial cells [29–31]. Essentially, PlGF is found in high amounts in the placenta, but it is also expressed at a low level under normal physiological conditions in several other organs including heart, lung, skeletal muscle, and adipose tissue [34–40].

The proangiogenic activity of members of the VEGF family of proteins, including PlGF, is achieved through the binding and activation of tyrosine kinase receptors [41, 42]. The most important receptors, which were found to bind the VEGF family of proteins with high affinity, are the fms-like tyrosine kinase receptor (Flt-1, also referred to as VEGF receptor 1, VEGFR1) and kinase domain region (KDR or VEGFR2) [43, 44]. These receptors are made up of a single signal sequence, a transmembrane domain, 7 immunoglobulin-like domains in their extracellular domain (the ligand-binding domain), and an intracellular tyrosine kinase domain [45].

However, it was noted that a cDNA in the endothelial cells of the human umbilical vein in the placenta encodes a truncated form of Flt-1 which is generated through alternative splicing of the mRNA. This soluble isoform of Flt-1 (sFlt-1) lacks the seventh immunoglobulin-like domain, the cytoplasmic domain, and the transmembrane sequence [45].

3.1.1. PlGF and sFlt-1 in Disease States and Pregnancy. One of the most important properties of vascular endothelial cells is their ability to proliferate and form a network of capillaries through a process termed angiogenesis [33]. In a normal adult, the angiogenic process is tightly regulated and is limited to the endometrium and the ovary during the different phases of the menstrual cycle, and to the heart and skeletal muscles following injury due to prolonged and sustained physical exercise [134]. This process is especially prominent during embryonic development (Ramsey & Donner (1980) and Gilbert (1988) as cited in [33]) since angiogenesis is essential for correct development of the embryo and for postnatal growth [134].

The complex interplay between some members of the VEGF family of proteins, including PlGF, and their cognate receptors, especially Flt-1, is essential for angiogenesis to occur [2]. On the other hand, the soluble splice variant of Flt-1, sFlt-1, is secreted into the circulation and acts as an antiangiogenic factor since it antagonises and neutralises PlGF and VEGF by binding to them and inhibiting their interaction with endothelial receptors on the cell surface [36, 45, 57].

PlGF is present during early embryonic development and throughout all the stages of pregnancy since it is highly expressed by the placenta. It has been suggested that the presence of this proangiogenic factor serves as a control for trophoblast growth and differentiation [31, 37], which in turn implies that PlGF has a role in the invasion of the trophoblast into the maternal decidua [135]. Concurrently, although sFlt-1 is secreted in small amounts by endothelial cells and monocytes, the placenta seems to serve as the major source of sFlt-1 in the circulation during pregnancy. This finding is emphasised by the significant fall in the level of circulating sFlt-1 following the delivery of the placenta [47].

In a normotensive pregnant woman, the level of PlGF in the maternal circulation increases gradually during the first two trimesters and peaks at midgestation, before declining again as the pregnancy comes to term. Alternatively, the sFlt-1 level in normotensive pregnant women remains relatively stable during the first two trimesters, after which it increases steadily until term [45, 47, 53]. This gestational variation can be observed in the results presented in the charts below which were obtained in the Prospective Multicenter Study: Diagnosis of Preeclampsia (Roche Study no. CIM RD000556/X06P006). In this study, the PlGF and sFlt-1 levels were measured in normotensive women from countries across Europe, who had singleton pregnancies and went on to have normal pregnancy outcomes (no PE/HELLP and no IUGR) (Figures 3 and 4).

The levels of these biomarkers have also been investigated in the maternal circulation of patients with PE. There is strong evidence for the reduced occurrence of free, bioactive PlGF, together with higher placental expression of sFlt-1 and, consequently, elevated levels of circulating sFlt-1 in preeclamptic patients during active disease when compared with normotensive pregnant women [46, 47, 50, 53, 55, 67].

In a large cross-sectional study comparing gestational age-matched women with active PE and normotensive pregnancies, PlGF levels were noted to be lower and sFlt-1

Table 2: List of proposed serum biomarkers for the detection and diagnosis of PE.

Proposed biomarker	Biological role	Serum level in PE compared to normotensive pregnancy	Type of study	Positive predictive value	References
Soluble fms-like tyrosine kinase 1 (sFlt-1)	Anti-angiogenic factor	Higher	Case-control	No	[24, 46–51]
			Nested case-control	No	[52, 53]
			Cross-sectional case-control	No	[54–56]
			Longitudinal case-control	No	[57, 58]
			Prospective cohort	No	[59, 60]
				Yes	[61]
			Prospective nested case-control	No	[62]
Placental growth factor (PlGF)	Angiogenic factor	Lower	Case-control	No	[24, 46, 48, 50, 51]
				Yes	[63–65]
			Nested case-control	No	[52, 53, 66]
			Cross-sectional case-control	No	[54–56]
			Longitudinal case-control	No	[57, 58, 67]
				Yes	[68]
			Prospective cohort	No	[59, 60]
				Yes	[61]
			Prospective nested case-control	No	[62]
			Prospective longitudinal case-control	No	[69]
			Longitudinal cohort	No	[70]
			Longitudinal cross-sectional	No	[71, 72]
Asymmetric Dimethyl-Arginine (ADMA)	Biochemical degradation product	Higher	Case-control	No	[73–77]
			Longitudinal case-control	No	[78–82]
			Cross-sectional case-control	No	[83, 84]
Soluble Endoglin (sEng)	Modulator of transforming growth factor (TGF)-β signalling	Higher	Longitudinal case-control	No	[58]
			Cross-sectional case-control	No	[85, 86]
			Nested case-control	No	[87, 88]
			Retrospective	No	[89]
			Prospective	No	[90]
			Case-control	No	[91]
Placental Protein 13 (PP-13)	Lysophospholipase activity	Higher	Longitudinal case-control	No	[92, 93]
			Nested case-control	No	[94–98]
			Case-control	No	[99]
P-Selectin	Calcium-dependent receptor	Higher	Cross-sectional case-control	No	[100–105]
				Yes	[106]
			Longitudinal case-control	No	[107]
				Yes	[108]
Adrenomedullin	Vasodilator	Higher	Cross-sectional case-control	No	[109]

Table 2: Continued.

Proposed biomarker	Biological role	Serum level in PE compared to normotensive pregnancy	Type of study	Positive predictive value	References
A Disintegrin and Metalloprotease 12 (ADAM12)	Cell-cell and cell-matrix interaction protease	Lower	Retrospective case-control	No	[110]
			Cross-sectional case-control	No	[111, 112]
Pentraxin 3 (PTX3)	Angiogenesis and inflammation factor	Higher	Cross-sectional case-control	No	[113, 114]
Pregnancy-Associated Plasma Protein A (PAPP-A)	Metalloproteinase that cleaves insulin-like growth factor binding proteins (IGFBPs)	Lower	Case-control	Yes	[64]
			Nested case-control	No	[98]
			Cross-sectional case-control	No	[115–118]
			Retrospective cohort	No	[119–122]
			Prospective cohort	No	[123]
Nicotinamide Phosphoribosyltransferase; Visfatin	Enzyme involved in nicotinamide metabolism	Both	Cross-sectional case-control	No	[124]
Cell free DNA	N.A	Higher	Cross-sectional case-control	No	[125–127]
Cell-free foetal DNA	N.A.	Higher	Cross-sectional case-control	No	[127–129]
			Nested case-control	No	[130, 131]
			Prospective	No	[132, 133]

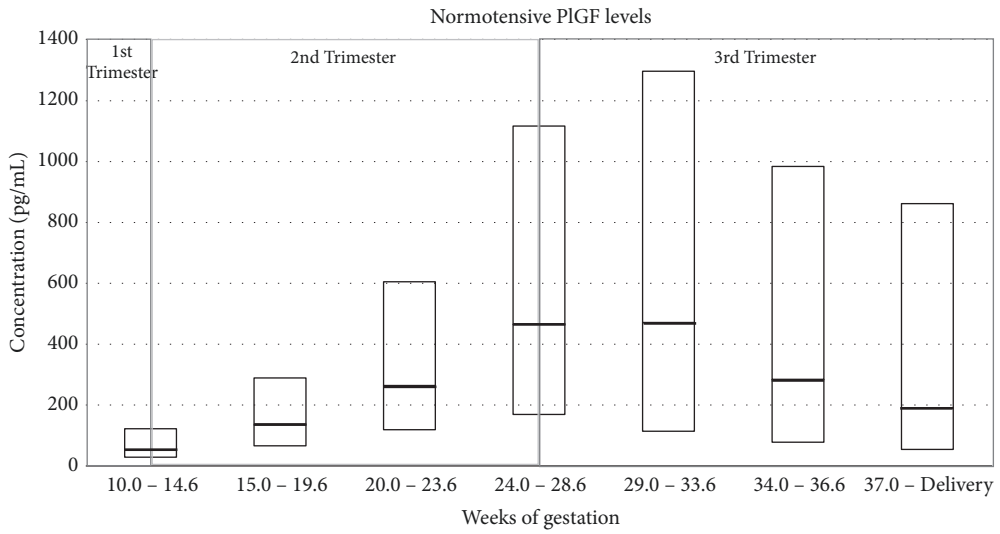

Figure 3: PlGF levels (pg/mL) measured in normotensive women during different weeks of gestation (based on data from Roche Study no. CIM RD000556/X06P006).

levels to be higher in the preeclamptic group (mean PlGF level of 137pg/mL versus 669pg/mL; mean sFlt-1 level of 4382pg/mL versus 1643pg/mL) [53]. The decrease in PlGF levels is thought to be due to the increased concentration of circulating sFlt-1 from 33 to 36 weeks of gestation and hence increased binding of PlGF to sFlt-1, rather than the decrease in PlGF caused by reduced production of PlGF [53].

In 2003, Maynard et al. introduced exogenous sFlt-1 into pregnant rats, and remarkably this led to reduced levels of PlGF, hypertension, and proteinuria, symptoms parallel to those observed in patients with PE [47]. This finding led to the idea that the maternal endothelial dysfunction that is noted in preeclamptic patients is caused by the imbalance of the levels of pro- and antiangiogenic factors in the maternal

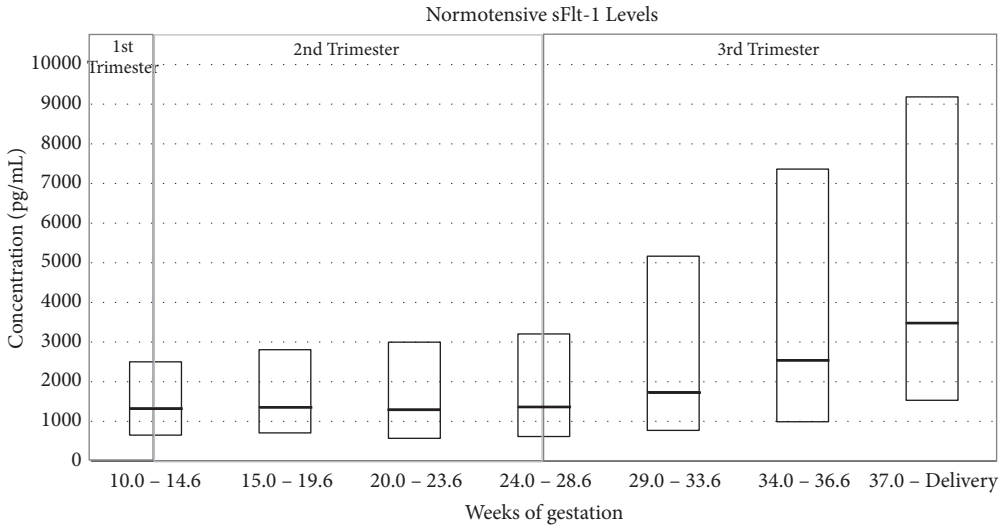

Figure 4: sFlt-1 levels (pg/mL) measured in normotensive women during different weeks of gestation (based on data from Roche Study no. CIM RD000556/X06P006).

circulation. There is much supportive evidence suggesting that the antagonism of PlGF by sFlt-1 may be responsible for the endothelial dysfunction in PE [59, 60].

The PlGF deficiency and sFlt-1 excess observed in preeclamptic patients may also be due to the placental hypoxia that is associated with incomplete remodelling of the maternal spiral arteries. This defective placentation, as a result of incompletely remodelled arteries, offers persistently high resistance to uterine artery blood flow, which may in turn predispose to vascular rupture in the placental bed, especially after the onset of hypertension [136, 137]. However, more evidence is required to determine whether the altered levels of these pro- and antiangiogenic factors are the consequence or the cause of the placentation defect in women with PE.

Studies have shown that the level of maternal PlGF was more significantly reduced in patients with severe symptoms of PE compared to normotensive pregnant women and women with symptoms of mild PE. On the other hand, in the case of maternal sFlt-1 levels, the increased levels were shown to correlate with the severity of PE, with mean sFlt-1 levels ranging from 1.50 ± 0.22ng/mL in normotensive pregnant women to 3.28 ± 0.83ng/mL in women with mild PE and to 7.64 ± 1.5ng/mL in women with severe PE [47, 48]. Furthermore, it has been noted that the variation in PlGF and sFlt-1 is more pronounced in early onset PE when compared to late onset PE as well as in women who had PE and later delivered small for gestational age (SGA) newborns [53, 56]. The results obtained by Levine et al. [53] which show these differences are presented in Figures 5 and 6.

In the study by Levine et al., it was also reported that the increase in sFlt-1 levels in the circulation of patients with PE corresponds to a decrease in free PlGF [53]. Moreover, it was also observed that the alterations in the levels of these factors precede the clinical diagnosis by several weeks. In fact, a significant finding in this study was that the elevated level of sFlt-1 can be detected in the maternal serum 5 weeks before the clinical symptoms of PE appear while the decreased PlGF level can be detected from 13 to 16 weeks of gestation in women who subsequently develop PE. This finding was later observed by a number of other studies [20, 53, 58, 62–66, 68–72, 138].

These findings have suggested that the measurement of PlGF and sFlt-1 may be used to predict the development of PE several weeks before the clinical onset of symptoms of this disease (Figure 7). The combined measurement of PlGF and sFlt-1 also distinguished women who subsequently developed PE from women who subsequently developed gestational hypertension, delivered SGA newborns, or completed a normal, healthy full term pregnancy [47, 52].

According to some studies, altered levels of sFlt-1 are specific for PE since no changes are detected in women who subsequently delivered SGA newborns or whose pregnancies were complicated by IUGR when compared to normotensive women with normal pregnancy outcomes [51, 58]. However, in a selected group of patients with abnormal uterine perfusion with subsequent IUGR, other studies have detected similar alterations in PlGF and sFlt-1 levels during the second trimester [139].

The combination of sFlt-1 and PlGF values in the form of a ratio, as shown for the Prospective Multicenter Study: Diagnosis of Preeclampsia (Roche Study no. CIM RD000556/X06P006) (Figure 8), has also been used as a predictor of PE. In a prospective study by Rana et al., [61] it was suggested that the ratio of sFlt-1 to PlGF appears to be a better predictor of PE than either measure alone. Kim et al. [24] revealed that the sFlt-1 to PlGF ratio in preeclamptic women was significantly higher when compared to the normal controls since the median value for the log [sFlt-1/PlGF] ratio in preeclamptic women was 1.6 (range 1.0 – 2.9), while the median value in the normotensive controls was 1.2 (range 0.5 – 1.9). In this study, a cut-off value of 1.4 was used since this showed 80.4% sensitivity and 78% specificity, with women having maternal log [sFlt-1/PlGF] ratio values more than 1.4 being at a higher risk of developing PE. Therefore, this ratio is a reliable marker

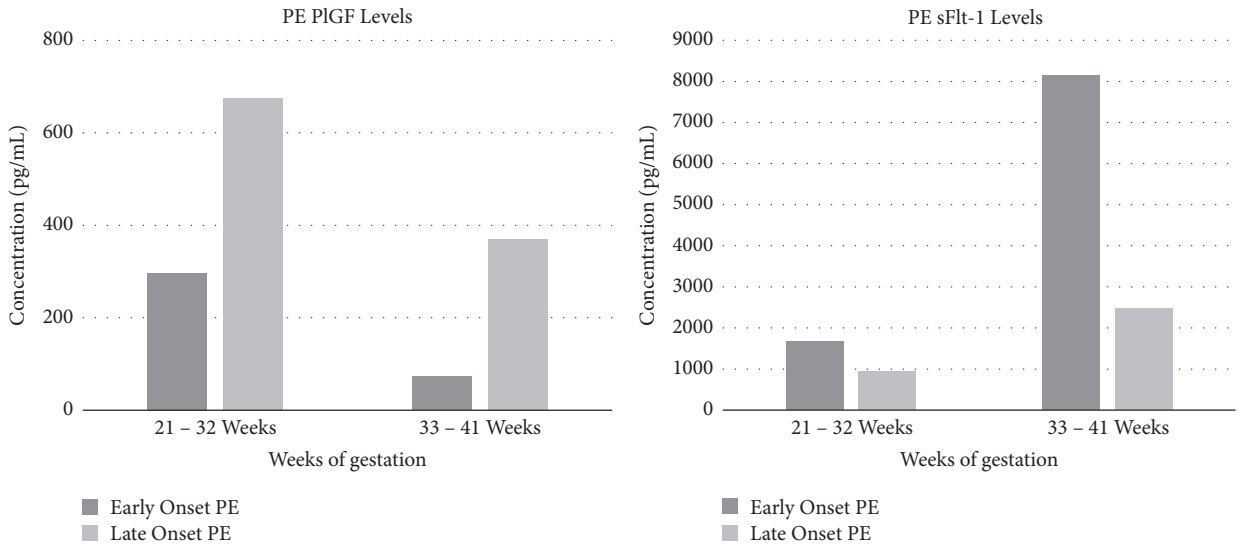

Figure 5: PlGF and sFlt-1 results obtained in women with early onset and late onset PE during different gestational periods [53].

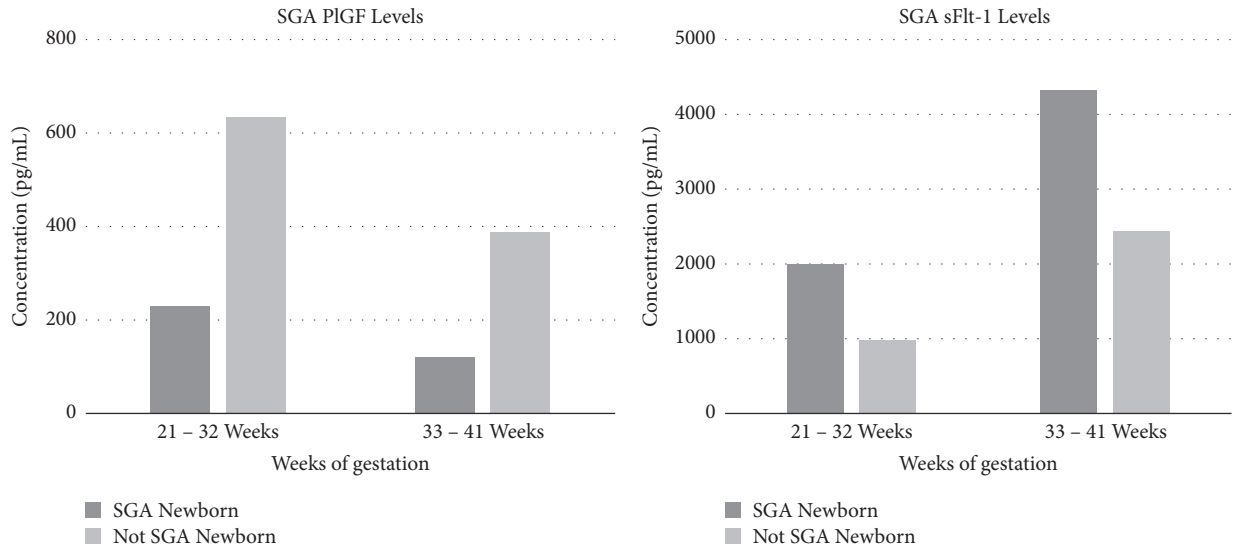

Figure 6: PlGF and sFlt-1 results obtained during different gestational periods in women with PE who later delivered small for gestational age (SGA) newborns and those that delivered infants of normal gestational size [53].

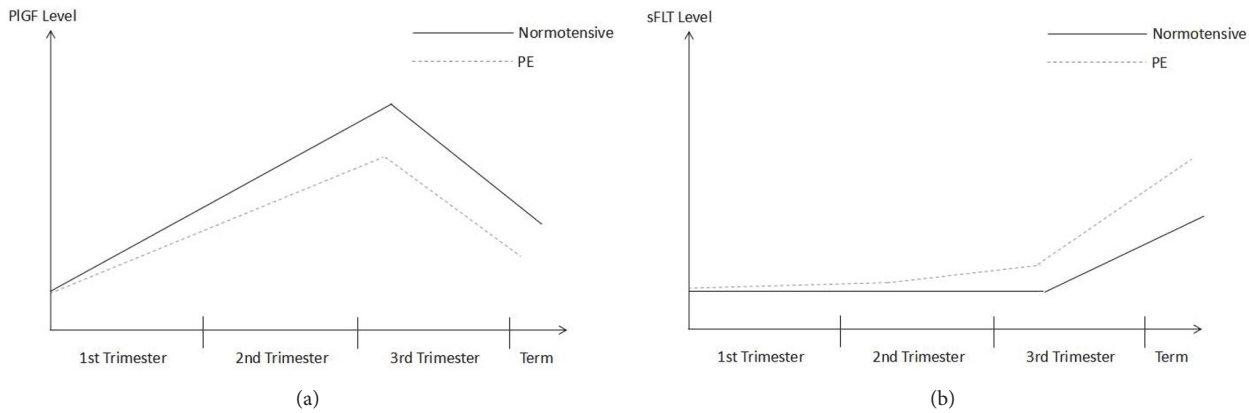

Figure 7: Levels of (a) PlGF and (b) sFlt throughout normotensive pregnancy as compared to levels in preeclamptic pregnant women.

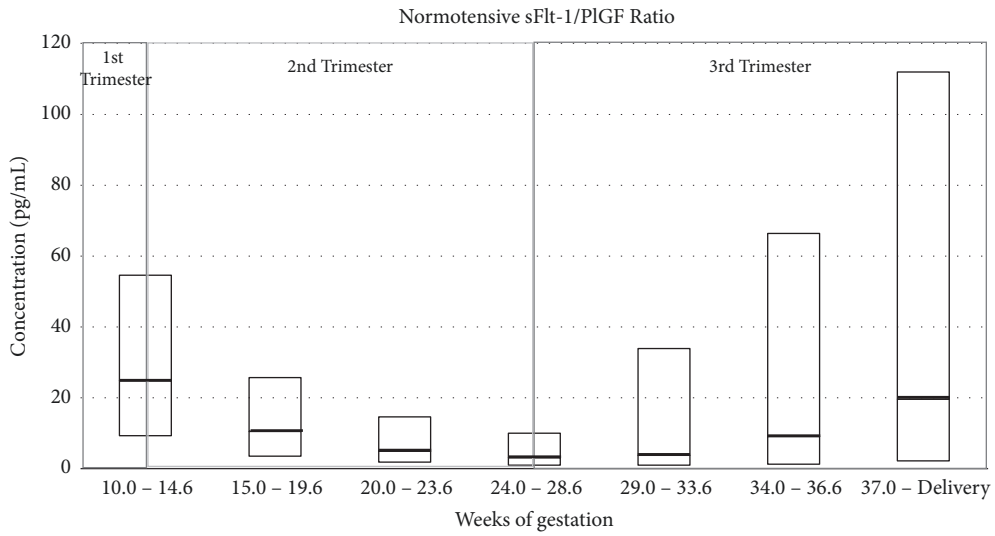

Figure 8: PlGF to sFlt-1 ratio (pg/mL) measured in normotensive women during different weeks of gestation (based on data from Roche Study no. CIM RD000556/X06P006).

of overall risk of PE and it may be used to distinguish between normal pregnancy and pregnancy complicated by PE and to define the severity of PE [2, 140].

The measurement of PlGF and sFlt-1 has only rarely been extended to the infants born from preeclamptic pregnancies. In 2005, Staff et al. measured PlGF and sFlt-1 levels in normotensive and preeclamptic pregnant women and their newborns [49]. The results obtained for the mothers reflected the same results obtained by studies mentioned in previous sections, with lower PlGF and higher sFlt-1 levels being noted in the preeclamptic group. In this study, the umbilical samples obtained from all newborns had PlGF levels that were below the concentration of the lowest standard of the ELISA kit used in the study (15.6pg/mL) and thus comparison between the preeclamptic and normotensive control groups could not be achieved. On the other hand, the median sFlt-1 concentration obtained for foetuses born to mothers with PE was found to be significantly higher than the median concentration obtained for those born to normotensive mothers (246 pg/mL, 95% CI for the median 163–255 versus 163 pg/mL, 95% CI for the median 136–201).

At the same time, although sFlt-1 levels were noted to be higher in foetuses born to mothers with PE, the sFlt-1 concentrations measured in umbilical samples were noted to be very low when compared to the maternal sFlt-1 concentrations. This finding suggests that the foetus does not contribute significantly to the elevated maternal sFlt-1 concentration in PE, which further reinforces the assumption that the increase in circulating sFlt-1 concentration in mothers with PE originates primarily from the placenta [49]. This finding is also consistent with the idea that foetuses do not experience hypertension or proteinuria like their preeclamptic mothers because they are not exposed to high concentrations of antiangiogenic factors, including sFlt-1, which, although of placental origin, should be primarily restricted to the maternal vasculature [141].

3.2. Protein Methylation Products. Protein methylation is a posttranslational modification (PTM) that involves the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to a particular protein residue under the control of specific methyltransferase enzymes [142]. This results in the generation of a methylated substrate and the by-product, S-adenosyl-L-homocysteine (SAH), which is then degraded by the enzyme S-adenosylhomocysteine hydrolase to give adenosine and homocysteine [143] (Figure 9). Such PTMs predominantly target the side chains of arginine and lysine, but other amino acid residues, including histidine, asparagine, glutamine, and cysteine, have been shown to serve as minor targets for methylation.

3.2.1. Asymmetric Dimethylarginine (ADMA). Different types of methylarginine are synthesised following arginine methylation, which is a PTM of the nitrogen atom forming part of the guanidino moiety of the arginine (R) group within proteins. Proteins that undergo arginine methylation are involved in a number of different cellular processes, including transcriptional regulation, RNA metabolism, and DNA damage repair [144]. This process involves the addition of one or two methyl groups, derived from S-adenosylmethionine (SAM) [145], to the guanidino nitrogen atom of arginine and is achieved with the help of protein arginine N-methyltransferase enzymes (PRMTs) which belong to a sequence-related family of methyltransferases [146]. The guanidino group of arginine can be methylated in three different ways to give ω-NG-monomethylarginine (MMA), ω-NG,N'G-symmetric dimethylarginine (SDMA), or ω-NG,NG-asymmetric dimethylarginine (ADMA) [144] (Figure 10).

ADMA is eliminated in part by urinary excretion, but it is mainly metabolised via hydrolytic degradation to citrulline and dimethylamine. This metabolic reaction is catalysed by the enzyme NG-dimethylarginine dimethylaminohydrolase

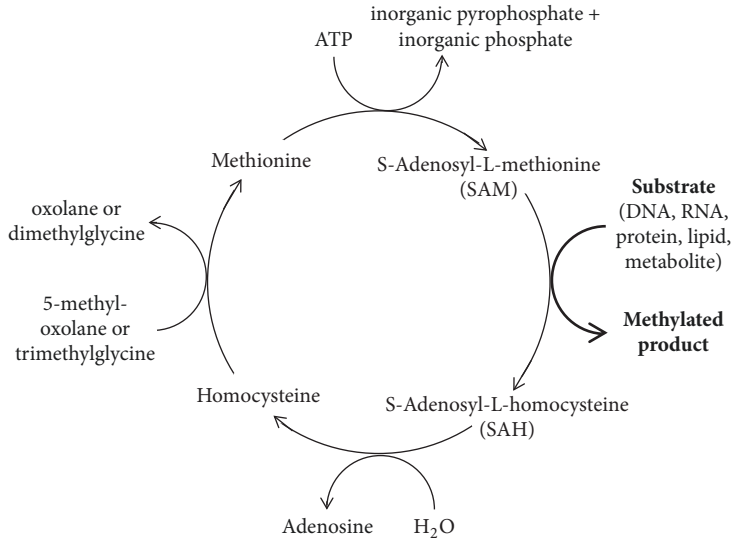

Figure 9: The S-adenosyl methionine cycle.

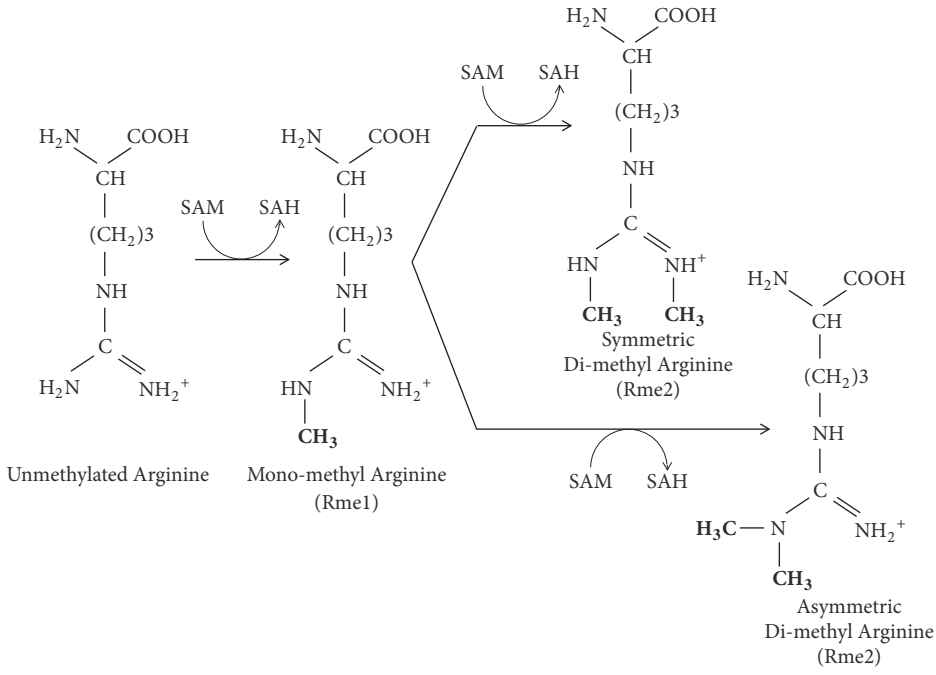

Figure 10: Formation of mono-, symmetrical, and asymmetrical dimethylarginine.

(DDAH) [147] (Figure 11). There are 2 isoforms of DDAH: DDAH-1 and DDAH-2. Tissues expressing neuronal nitric oxide synthase (NOS) usually contain DDAH-1, while tissues containing the endothelial isoform of NOS (eNOS) predominantly contain DDAH-2 [148]. Thus, it has been observed that DDAH-1 is found in high levels in the kidneys and liver, whereas DDAH-2 is the most abundant isoform in the endothelium [145].

(1) ADMA in Disease States and Pregnancy. In 1992, it was reported that ADMA is an endogenous competitive inhibitor of NOS [149]. NOS is responsible for the synthesis of nitric oxide in endothelial cells since it catalyses the conversion of L-arginine to L-citrulline and NO [150]. ADMA is an analogue of L-arginine which is also synthesised and released by endothelial cells.

NO plays multiple roles in the cardiovascular system [144]. It is a potent vasoactive mediator that is released in response to stress [151] and is important in maintaining endothelial homeostasis [145]. Apart from inducing vasodilatation to regulate vascular tone and tissue blood flow [150–152], endothelial NO also inhibits platelet aggregation

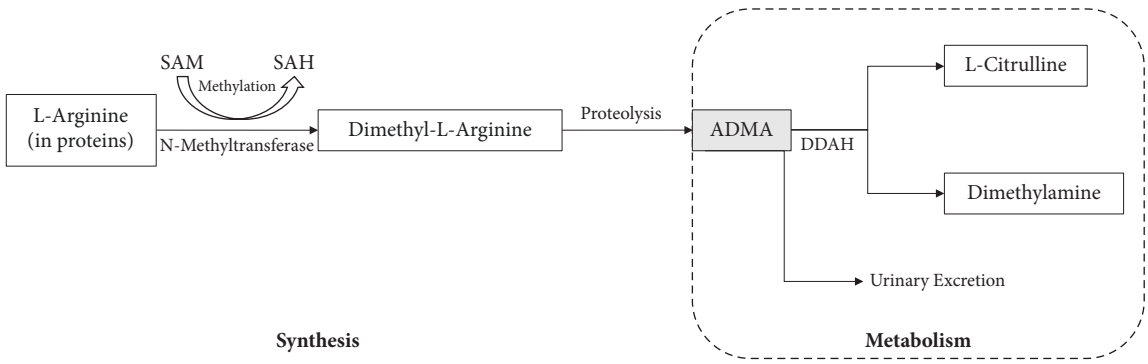

Figure 11: Overview of the synthesis and metabolism of ADMA. Synthesis of ADMA involves the methylation of arginine residues with the help of N-methyltransferase (protein arginine N-methyltransferases, PRMTs) which converts the methyl donor S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH) followed by proteolytic breakdown of the proteins, which generates ADMA and N-monomethyl-L-arginine (L-NMMA). Elimination of ADMA is partly achieved via urinary excretion. However, ADMA is mainly eliminated through its metabolism to citrulline and dimethylamine by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) [145].

[153], inhibits adhesion of leukocytes and monocytes to the endothelium [154], and inhibits smooth muscle cell proliferation [155].

It has been noted that decreased levels or inhibition of DDAH, which is the enzyme that catalyses the hydrolysis of ADMA, results in higher levels of ADMA in the circulation and causes gradual vasoconstriction [156]. This occurs because the elevated level of ADMA in the circulation results in the reversible inhibition of endogenous NO synthesis which in turn could lead to endothelial dysfunction [157]. The low levels of NO result in increased systemic vascular resistance and blood pressure [75]. High levels of ADMA have been observed in individuals with cardiovascular diseases including atherosclerosis, hypertension, and hypercholesterolaemia and in individuals with chronic renal failure [145]. Conventional cardiovascular risk factors may reduce DDAH activity by increasing oxidative stress, and this will in turn also result in elevated levels of ADMA [148, 158–160].

In a study in 1998 by Holden et al. [73], it was determined that pregnant women have a lower concentration of ADMA in their circulation than nonpregnant women. Their findings revealed that while the mean ADMA concentration in nonpregnant women was 0.82 ± 0.31μmol/L, the mean values of ADMA in pregnant women were in the range of 0.40 ± 0.15 μmol/L in the first trimester, 0.52 ± 0.20μmol/L in the second trimester, and 0.56 ± 0.22μmol/L in the third trimester. A similar observation was later made by Maeda et al. [79] who also noted lower mean ADMA concentrations in pregnant women (0.29 ± 0.05μmol/L in the first and third trimesters and 0.32 ± 0.05μmol/L at term) when compared to mean ADMA levels in nonpregnant women (0.41 ± 0.06μmol/L). At the same time, the results obtained by Holden et al. revealed that although the mean ADMA levels are lower in pregnant women, these tend to increase during the normal gestational period [73]. This finding is not reflected in the results obtained by Maeda et al. since the latter group did not note a change in mean ADMA levels from the first to the third trimester (0.29 ± 0.05μmol/L in the first and third trimesters), with the only increase being noted at full term (0.32 ± 0.05μmol/L at term) [79]. Alternatively, the increase

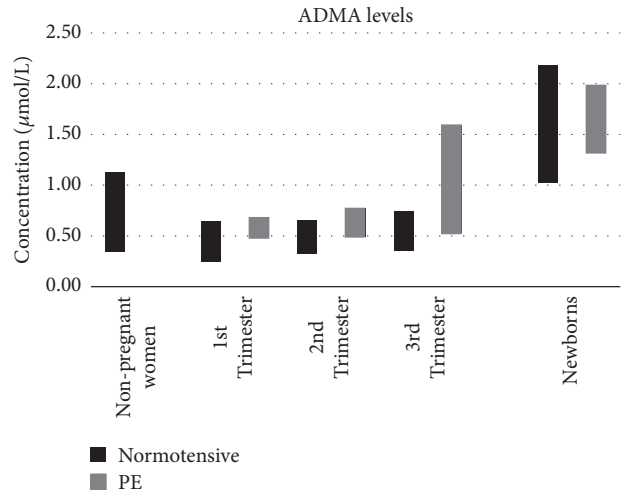

Figure 12: Levels of ADMA throughout pregnancy as compared to levels in nonpregnant women.

in mean ADMA levels during pregnancy was observed later on in a study by Rizos et al. in 2012 who showed that the mean ADMA levels in pregnant women increased from 0.51 ± 0.14μmol/L in the first trimester to 0.52 ± 0.13μmol/L in the second trimester and finally to 0.58 ± 0.16μmol/L in the third trimester [81] (Figure 12). Such findings have suggested that ADMA may have a role in vascular dilation and blood pressure regulation during pregnancy [73].

Numerous studies have measured the level of ADMA in pregnant women to determine whether there is a significant difference in ADMA concentrations in the circulation of women with PE when compared to women with uncomplicated pregnancies.

Discrepant findings have been observed. In separate studies in 1998, both Holden et al. [73] and Pettersson et al. [80] observed elevated mean ADMA levels during the third trimester in preeclamptic patients (1.17 ± 0.42μmol/L and 0.55 ± 0.02μmol/L, respectively) when compared to the normotensive pregnant controls during the same gestational

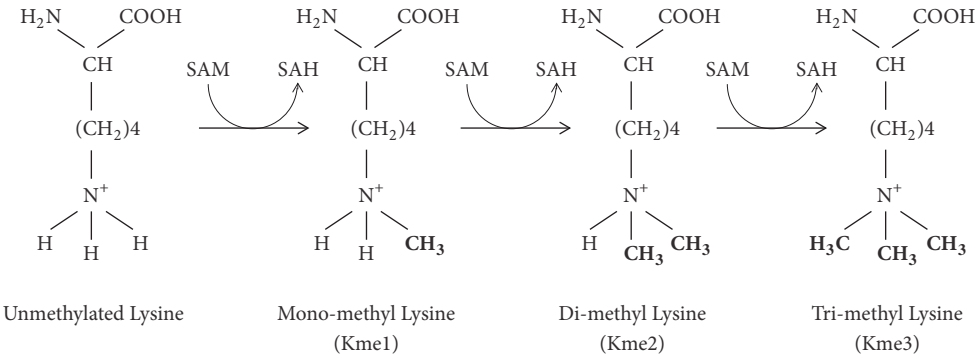

Figure 13: Formation of mono-, di-, and trimethyl-lysine.

period (0.56 ± 0.22μmol/L and 0.36 ± 0.01μmol/L, respectively). Similarly, the study by Rizos et al. [81] also showed elevated mean ADMA levels during all three trimesters in preeclamptic patients (0.58 ± 0.10μmol/L in the first trimester, 0.63 ± 0.14μmol/L in the second trimester, and 0.68 ± 0.11μmol/L in the third trimester) compared to women with uncomplicated pregnancies (0.51 ± 0.14μmol/L in the first trimester, 0.52 ± 0.13μmol/L in the second trimester, and 0.58 ± 0.16μmol/L in the third trimester). However, in a number of other studies, although the median ADMA levels demonstrated a similar increased trend in preeclamptic patients, these findings were shown not to be statistically significant [74, 76, 77].

Furthermore, elevated ADMA concentrations have been noted in the circulation of pregnant women who went on to develop PE. This increased ADMA concentration was noted prior to the development of clinical signs and symptoms of PE [82, 83], which suggests that ADMA could have a role in the pathogenesis of this condition. Since nitric oxide is known to be important in maintaining both maternal and foetal blood flow and vascular tone and in maintaining the foetomaternal circulation, it has been proposed that elevated levels of ADMA in pregnancy, as well as the consequent decreased levels of NO in the circulation, may contribute to the pathophysiological features of PE [75, 79].

The measurement of ADMA levels in umbilical cord blood samples might be important to explain the regulatory mechanisms of the circulatory system during the perinatal period [84]. However, data regarding the level of ADMA in neonates is limited. In the previously mentioned study by Maeda et al. [79], it was also observed that the ADMA level measured in umbilical blood was significantly higher than the maternal level, which was noted to be highest at term (1.02 ± 0.18μmol/L versus 0.32 ± 0.05μmol/L, respectively). This finding was later observed by Tsukahara et al. [84], who noted that the ADMA levels measured in umbilical blood from control newborns (newborns born to normotensive mothers following uncomplicated pregnancies) were about two times higher than the ADMA levels measured in lactating women, healthy children, and healthy adults (1.71 ± 0.47μmol/L versus 0.71 ± 0.06μmol/L, 0.71 ± 0.11μmol/L, and 0.52 ± 0.12μmol/L, respectively).

When comparing ADMA levels measured in umbilical blood of control newborns and newborns born to mothers with PE, Tsukahara et al. [84] found no significant difference between the two since their respective mean values were 1.71 ± 0.47μmol/L and 1.66 ± 0.33μmol/L. However, in a recent study by Gumus et al. [78], the median values for ADMA were noted to be significantly higher in umbilical blood from newborns born to mothers with PE than those from the control newborns (8.344ng/L versus 4.603ng/L). It was also noted that the level of ADMA measured from the umbilical cord blood sample correlated with the severity of the preeclamptic disorder.

3.2.2. Methyl-Lysine. In the case of lysine methylation, specific protein lysine methyltransferases (KMTs) catalyse the transfer of one, two, or three methyl groups from SAM to the epsilon (ε)-amine group of the side chain of a particular lysine residue [142]. This results in the formation of different forms of methylated lysines, namely, monomethyl-, dimethyl-, and trimethyl-lysines, respectively [142] (Figure 13). Some protein KMTs are specific for one or two of these modifications while others may result in the formation of all three derivatives [161]. Thus, it has been shown that these enzymes express product specificity since the type of methyl-lysine that is produced depends on the particular enzyme catalysing the reaction [162].

Nine functional members of the PRMT family have been identified (PRMT1-9) and the specificity of these enzymes for protein substrates varies and is generally much broader than that of KMTs. For instance, it has been shown that PRMT1, 2, 3, 4, 6, and 8 catalyse asymmetric dimethylation of arginine residues while enzyme PRMT5 catalyses symmetric dimethylation and PRMT7 may only catalyse monomethylation [163–165].

Most methyltransferase enzymes are grouped according to their structural features into three large families, namely, seven beta (β) strand [166], SET (suppressor of variegation 3-9 (Su(var)3-9), enhancer of zeste (E(z)), and trithorax (Trx)) domain-containing [167], and SPOUT domain-containing [168] enzymes. However, while all PRMTs belong to the seven β strand family of enzymes, most of the KMTs contain a conserved SET domain [169], which harbours the enzymatic activity of these proteins [170], and hence belong to the SET domain-containing family [171, 172]. Furthermore, an increasing number of enzymes which belong to the seven β

strand family have been shown to catalyse similar methylation reactions [173–176]. Thus, KMTs can be broadly divided according to their enzymatic domain into SET domain-containing and non-SET domain-containing proteins.

The SET domain-containing KMTs have been classified into a number of families according to the sequence motifs surrounding the SET domain. Members of the same family share similar sequence motifs surrounding the SET domain and often also share a higher level of similarity in the SET domain. Seven main families are known and these include the suppressor of variegation (Su(var)) 3-9 (SUV39), SET1, SET2, enhancer of zeste (E(z)), retinoblastoma-interacting zinc-finger protein (RIZ), SET and Myeloid-Nervy-DEAF1 (MYND) domain-containing protein (SMYD), and suppressor of variegation (Su(var)) 4-20 (SUV4-20) families. These families are accompanied by SET7/9 and SET8 (also known as PR-SET7) which are SET domain-containing KMTs but do not fit in with the previously mentioned families [177]. A tabulated list of the KMTs found in humans which belong to each KMTs family, as well as SET7/9 and SET8, together with their properties has been presented by Dillon et al. [177].

Although a large majority of KMTs contain the SET domain, numerous other proteins which do not contain the SET domain, including the disruptor of telomeric silencing (DOT) 1-like (DOT1L) [169, 178] and methyltransferase-like (METTL) family proteins [179], also have lysine methyltransferase activity.

(1) Lysine Methylation in Disease States and Pregnancy. Along the years, numerous KMTs and lysine demethylases (KDMs) have been identified and their activity has been reported to be important in several biological processes, including the regulation of gene expression, cell-cycle progression, DNA replication, and differentiation [180–185]. In normal, healthy states, lysine methylation is tightly controlled and a balance in lysine methylation is maintained by the opposing actions of KMTs and KDMs [186]. At the same time, gene expression patterns must be able to respond to developmental requirements and environmental changes in order to maintain a healthy state [187].

The dysregulation of PTMs in the form of inappropriate expression (inclusion or elimination), as well as mutation of numerous KMTs and KDMs, may be a critical determinant of different diseases, including ageing and cancer [186–191]. In fact, the loss of this appropriate balance in methylation in adult stem cells has been thought to contribute to the decline of tissue function with age [192]. Studies have also shown that aberrant methylation is associated with an increased incidence of various types of cancers and poor survival [193, 194]. For instance, the methyltransferase responsible for histone 3 lysine 27 trimethylation (H3K27me3) is upregulated in prostate cancer [195], breast cancer [196], and lymphomas [197].

The human genome encodes over 200 methyltransferases [198] and although most studies have focused on histone methylation, a number of reports have revealed that these enzymes are also responsible for the regulation of methylation of nonhistone proteins [199]. It has been observed that a number of nonhistone proteins undergo methylation on their lysine residues and this in turn leads to changes in the function and/or stability of these nonhistone proteins [199, 200]. Evidence of lysine methylation-dependent regulation for an ever-increasing number of nonhistone proteins has been reported and in some cases these changes would also be of relevance to stress, hypertension, and PE as described below.

Lysine Methylation of p53. The tumour suppressor protein p53 functions as a sequence-specific transcription factor which regulates important cellular processes including cell-cycle arrest, DNA repair, apoptosis, and senescence in response to stress signals. Under normal conditions, the level of p53 in the cell is maintained low; however, p53 is rapidly stabilised and activated in response to cellular stresses such as DNA damage and hypoxic states [143].

Trophoblast apoptosis in the human placenta has been shown to increase during the gestational period [201]. Furthermore, in pregnancies complicated by PE, dysregulation of cell turnover, which results in increased apoptosis [202–204] and reduced syncytiotrophoblast area [205], has been noted. The impact of exaggerated apoptosis on the placental pathology in cases of PE is unclear; however, this may ultimately prevent the replenishment of the syncytiotrophoblast, promote degeneration of the syncytium, and result in the release of vasoactive or inflammatory factors into the maternal circulation [206]. Since p53 is a vital regulator of the apoptotic pathway, its level has been measured in cases of PE and it has been observed that, at the protein level, the level of p53 is significantly elevated in placentas obtained from pregnancies complicated by PE [207]. This increase in p53 expression was also noted in cases of foetal IUGR [208, 209]. Also, the increase in p53 levels was associated with an increased expression of downstream elements of the apoptotic pathway, including the level of the downstream effector protein p21 [207].

Methylation of p53 by SET7 (KMT7) was the first KMT-mediated methylation of a nonhistone protein reported [210]. Since then, a number of other KMTs, including SET9 (KMT5), SMYD2 (KMT3C), and SET 8 (KMT5A), which methylate p53 at specific C-terminal lysines, together with the lysine-specific demethylase KDM1(LSD1) which mediates p53 demethylation, have also been identified [143] (Figure 14).

P53 undergoes multiple PTMs, including lysine methylation, which regulate its stability, protein-protein interactions, and transcriptional activity. In fact, the transcriptional activity of p53 is enhanced or suppressed depending on the methylation site. Also, the interaction of p53 with its coactivator p53 binding protein 1 (53BP1) to induce apoptosis is mediated through the action of the lysine demethylase KDM1. The balance between methylation and demethylation, in combination with other PTMs, is essential in the response of p53 to cellular stresses since its activity is important in the prevention of tumour formation [143].

Lysine Methylation of Heat Shock Protein (HSP) 70. HSPs are primarily known as intracellular proteins that have molecular chaperone and cytoprotective functions [211] and

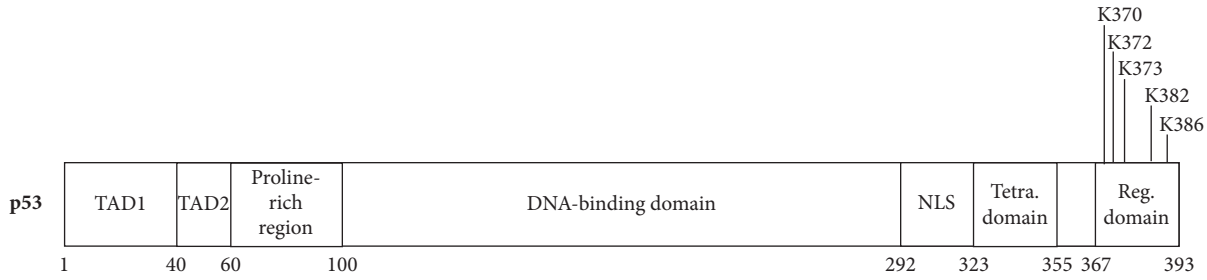

Figure 14: p53 lysine methylation sites (based on PhosphoSite data).

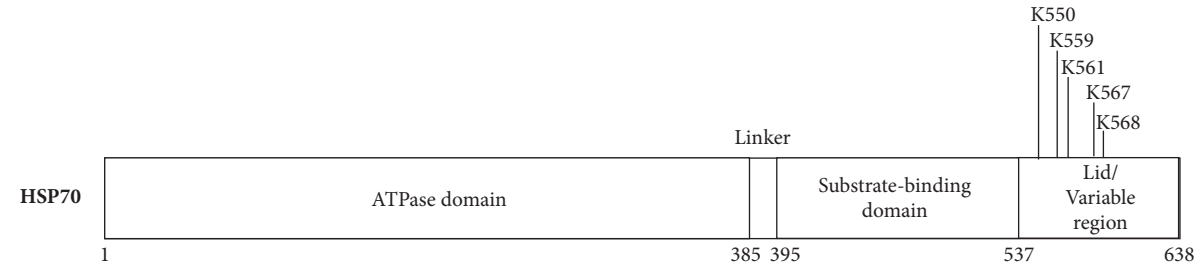

Figure 15: HSP70 lysine methylation sites (based on PhosphoSite data).

are essential for cell recovery, survival, and maintenance of homeostasis [212]. HSP70 proteins are ubiquitous, adenosine triphosphate- (ATP-) dependent molecular chaperones which make up one of the most evolutionarily conserved family of proteins [213]. Extracellular HSP70 may contribute to the development of autoimmune disease and may provide an indication of the status of the innate immune system [214–216]. In humans, these proteins are encoded by 13 genes and they are either induced in response to stress (such as heat shock) or constitutively expressed [217]. HSP70 proteins carry out numerous biological processes including protein-protein interactions, protein degradation, and translocation across membranes [218].

HSP70 has been shown to be elevated in cases of PE [212, 219]. Park et al. [220] suggested that increased levels of systemic HSP70 in preeclamptic patients originate from syncytiotrophoblasts and villous endothelial cells of preeclamptic placentas since these were shown to have higher HSP70 proteins levels when compared to placentas from normal, healthy pregnancies. Other studies have also detected higher HSP70 levels in placental tissues of preeclamptic patients [221, 222]. This increase in serum HSP70 reflects systemic inflammation and oxidative stress that is noted in PE [212, 219]. Initially, expression of HSP70 plays a protective role against placental oxidative stress; however, the overexpression of HSP70 may lead to intervillous endothelial dysfunction and may play a role in the pathogenesis of PE [220].

It has been shown that HSP70 can be posttranslationally modified in a number of ways, and numerous methylated lysines have been reported (Figure 15). For instance, it has been reported that the lysine residue K561, which is found in several human HSP70 proteins, can be methylated. Lysine methylation of HSP70 proteins is physiologically significant, especially in tumourigenesis. In particular, dimethylation of K561 of HSP70 by SETD1A regulates the subcellular localisation of this protein and it promotes the proliferation of cancer cells through its interaction with Aurora Kinase B (AURKB) [143, 223].

Lysine Methylation of Vascular Endothelial Growth Factor Receptor 1 (VEGFR-1) and 2 (VEGFR-2). As was described in previous sections, VEGFR-1 (or Flt-1) and VEGFR-2 are membrane receptors which belong to a receptor tyrosine kinase (RTK) subfamily. VEGRF-1 is able to bind tightly to its ligands, VEGF (including VEGF-A) and PlGF, but has weak tyrosine kinase activity and hence generates an overall weaker signal than other RTKs [224]. Through its interaction with its ligands, VEGFR-1 plays an important role in both physiologic and pathologic angiogenesis, the process by which new blood vessels are formed from preexisting vessels [43, 225, 226]. Angiogenesis is crucial for the normal physiological functions of tissues, but it is also important for the progression of certain diseases, including cancer and inflammation [227, 228].

VEGFR-1 has also been shown to be important in the regulation of vasculogenesis, which is the process by which new blood vessels are formed from precursor cells during early embryogenesis [225]. In 1995, Fong et al. [229] demonstrated that mutant mice which do not express VEGFR-1 (Flt-1-null, Flt-1-/- mice) die in utero due to the uncontrolled growth of vascular endothelial cells and disorganisation of blood vessels, which indicates that VEGFR-1 may have a negative regulatory role in angiogenesis during early embryogenesis.

VEGFR-1, together with its ligand VEGF and the other receptors for VEGF, is expressed throughout the gestational period in the placenta and these are essential for embryonic vascular development [34, 35]. During normal, uncomplicated pregnancies, VEGFR-1 can be primarily detected in

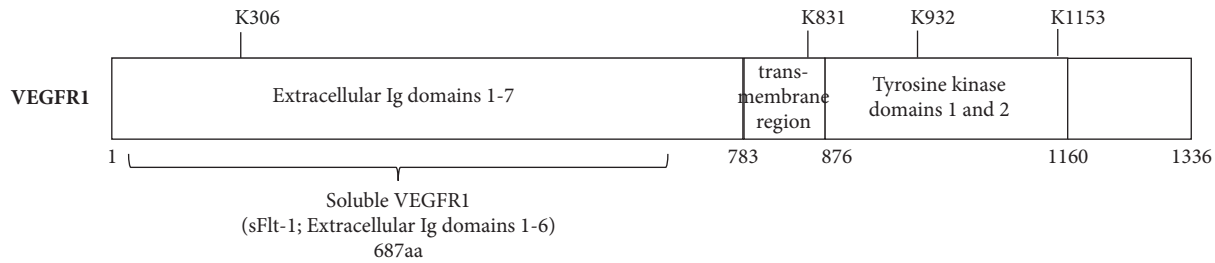

Figure 16: VEGFR1 lysine methylation sites (based on PhosphoSite data).

placental syncytiotrophoblasts [230]. Altered levels of VEGF and its receptors during pregnancy can lead to a disruption in angiogenesis which results in placental insufficiency and endothelial dysfunction, both of which are noted in pregnancies complicated by PE [231]. In the study by Helske et al. [230], the expression of VEGFR-1 was seen to be increased in the syncytiotrophoblasts of placentas obtained from a number of cases of PE, but not in all.

VEGFR-1 undergoes methylation at multiple lysine residues and it has been observed that this methylation is important in the regulation of the activity of VEGFR-1 (Figure 16). For instance, it has been shown that VEGFR-1 is a nonhistone target of SMYD3 methyltransferase since lysine 831 of VEGFR-1 is methylated by SMYD3 in vitro [232]. This methylation of VEGFR-1 enhances its kinase activity since lysine 831 is located within the kinase domain of this RTK.

VEGFR-2 is a potent angiogenic RTK, thus making it one of the most important RTKs in endothelial cells [233, 234]. The activity of VEGFR-2 has been noted to be essential in both vasculogenesis and pathological angiogenesis during cancer and ocular neurovascularisation [226].

Consequently, the expression and function of VEGFR-2 are highly regulated since increased angiogenesis plays a significant role in the progression of cancer and other diseases, including age-related macular degeneration, whereas insufficient angiogenesis has been linked to coronary heart disease and delayed wound healing [234]. VEGFR-2 undergoes methylation at multiple lysine residues and it has been shown that this methylation is important in the regulation of VEGFR-2 activity [233]. In fact, Hartsough et al. [233] also determined that methylation of lysine 1043 is essential in controlling the activation of VEGFR-2 since this methylation reaction is important for the tyrosine phosphorylation of this RTK. Consequently, interference of this methylation of VEGFR-2 results in an inability of VEGFR-2 to stimulate angiogenesis.

4. Conclusion

As with all biomarkers, their effectiveness is determined by the ability to diagnose the disease well before the presentation of clinical symptoms, and the advantage is that the changes can be detected through the minimally invasive blood tests, performed as part of the routine checks. So far, PlGF and sFlt-1, alone or in combination, not only have shown promise for the early diagnosis of PE but also have been shown to correlate well to the severity of this condition, with data from different cohorts being comparable. Similarly, the screening for ADMA has produced interesting trends and can be considered a useful candidate biomarker also based on the knowledge of its role in NO biochemistry. Our laboratory is also interested in exploring lysine methylation in conjunction with the above-mentioned potential biomarkers in order to extend the understanding of the role played by protein methylation in normal and PE biochemistry. Aside from the biomarkers selected, the final aim is to improve pregnancy progression and outcome, as well as to reduce the risks for both mother and foetus. This can only be achieved by unravelling and better understanding the underlying mechanisms leading to the preeclamptic condition.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

References

[1] L. K. Wagner, "Diagnosis and management of preeclampsia," *American Family Physician*, vol. 70, no. 12, pp. 2317–2324, 2004.

[2] S. Grill, C. Rusterholz, R. Zanetti-Dällenbach et al., "Potential markers of preeclampsia - A review," *Reproductive Biology and Endocrinology*, vol. 7, article no. 70, 2009.

[3] A. Reddy, S. Suri, I. L. Sargent, C. W. G. Redman, and S. Muttukrishna, "Maternal circulating levels of activin A, inhibin A, sFlt-1 and endoglin at parturition in normal pregnancy and preeclampsia," *PLoS ONE*, vol. 4, no. 2, Article ID e4453, 2009.

[4] A. P. Mackay, C. J. Berg, and H. K. Atrash, "Pregnancy-Related Mortality from Preeclampsia and Eclampsia," *Obstetrics & Gynecology*, vol. 97, no. 4, pp. 533–538, 2001.

[5] K. Braekke, P. M. Ueland, N. K. Harsem, and A. C. Staff, "Asymmetric dimethylarginine in the maternal and fetal circulation in preeclampsia," *Pediatric Research*, vol. 66, no. 4, pp. 411–415, 2009.

[6] C. W. Redman and I. L. Sargent, "Latest advances in understanding preeclampsia," *Science*, vol. 308, no. 5728, pp. 1592–1594, 2005.

[7] B. SIBAI, G. DEKKER, and M. KUPFERMINC, "Pre-eclampsia," *The Lancet*, vol. 365, no. 9461, pp. 785–799, 2005.

[8] ACOG Committee on Practice Bulletins—Obstetrics, "ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia," *Obstetrics & Gynecology*, vol. 99, no. 1, pp. 159–167, 2002.

[9] E. J. Roccella, "Report of the national high blood pressure education program working group on high blood pressure in

pregnancy," *American Journal of Obstetrics & Gynecology*, vol. 183, no. 1, pp. S1–S22, 2000.

[10] S. Grima, M. Vella, and C. Savona-Ventura, "Hypertensive disorders during pregnancy in pre-gestational diabetic women," *Malta Medical Journal*, vol. 22, no. 2, pp. 15–18, 2010.

[11] A. Díaz Pérez, A. Roca Pérez, G. Oñate Díaz, P. Castro Gil, and E. Navarro Quiroz, "Interaction and dynamics of these risk factors in hypertensive disorders of pregnancy: a pilot study," *Salud Uninorte*, vol. 33, no. 1, pp. 27–38, 2017.

[12] G. Dekker and B. Sibai, "Primary, secondary, and tertiary prevention of pre-eclampsia," *The Lancet*, vol. 357, no. 9251, pp. 209–215, 2001.

[13] H. Lipstein, C. C. Lee, and R. S. Crupi, "A current concept of eclampsia," *The American Journal of Emergency Medicine*, vol. 21, no. 3, pp. 223–226, 2003.

[14] E. Nexø, "Clinical biochemistry," *FEBS Letters*, vol. 375, no. 3, pp. 312-313, 1995.

[15] K. Duckitt and D. Harrington, "Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies," *British Medical Journal*, vol. 330, no. 7491, p. 565, 2005.

[16] J. P. Warrington, E. M. George, A. C. Palei, F. T. Spradley, and J. P. Granger, "Recent advances in the understanding of the pathophysiology of preeclampsia.," *Hypertension*, vol. 62, no. 4, pp. 666–673, 2013.

[17] T.-H. Hung, J. N. Skepper, D. S. Charnock-Jones, and G. J. Burton, "Hypoxia-reoxygenation: A potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia," *Circulation Research*, vol. 90, no. 12, pp. 1274–1281, 2002.

[18] N. Soleymanlou, I. Jurisica, O. Nevo et al., "Molecular evidence of placental hypoxia in preeclampsia," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 7, pp. 4299–4308, 2005.

[19] J. Roberts, "Is oxidative stress the link in the two-stage model of pre-eclampsia?" *The Lancet*, vol. 354, no. 9185, pp. 788-789.

[20] U. D. Anderson, M. G. Olsson, S. Rutardttir et al., "Fetal hemoglobin and α1-microglobulin as first- and early second-trimester predictive biomarkers for preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 204, no. 6, pp. 520–e5, 2011.

[21] G. J. Burton and E. Jauniaux, "Oxidative stress," *Best Practice & Research Clinical Obstetrics & Gynaecology*, vol. 25, no. 3, pp. 287–299, 2011.

[22] K. May, L. Rosenlöf, M. G. Olsson et al., "Perfusion of human placenta with hemoglobin introduces preeclampsia-like injuries that are prevented by α1-microglobulin," *Placenta*, vol. 32, no. 4, pp. 323–332, 2011.

[23] A. H. Shennan, L. Poston, L. C. Chappell, and P. T. Seed, "Prevention of pre-eclampsia," *The Lancet*, vol. 357, no. 9267, p. 1534, 2001.

[24] S.-Y. Kim, H.-M. Ryu, J.-H. Yang et al., "Increased sFlt-1 to PlGF ratio in women who subsequently develop preeclampsia," *Journal of Korean Medical Science*, vol. 22, no. 5, pp. 873–877, 2007.

[25] R. Madazli, E. Budak, Z. Calay, and M. F. Aksu, "Correlation between placental bed biopsy findings, vascular cell adhesion molecule and fibronectin levels in pre-eclampsia," *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 107, no. 4, pp. 514–518, 2000.

[26] N. Hadker, S. Garg, C. Costanzo et al., "Financial impact of a novel pre-eclampsia diagnostic test versus standard practice: A decision-analytic modeling analysis from a UK healthcare payer perspective," *Journal of Medical Economics*, vol. 13, no. 4, pp. 728–737, 2010.

[27] U. D. Anderson, M. G. Olsson, K. H. Kristensen, B. Åkerström, and S. R. Hansson, "Review: biochemical markers to predict preeclampsia," *Placenta*, vol. 33, pp. S42–S47, 2012.

[28] P. von Dadelszen, L. A. Magee, and J. M. Roberts, "Subclassification of Preeclampsia," *Hypertension in Pregnancy*, vol. 22, no. 2, pp. 143–148, 2003.

[29] D. Maglione, V. Guerriero, G. Viglietto, P. Delli-Bovi, and M. G. Persico, "Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 88, no. 20, pp. 9267–9271, 1991.

[30] S. Hauser and H. A. Weich, "A heparin-binding form of placenta growth factor (plGF-2) is expressed in human umbilical vein endothelial cells and in placenta," *Growth Factors*, vol. 9, no. 4, pp. 259–268, 1993.

[31] D. Maglione, V. Guerriero, G. Viglietto et al., "Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14," *Oncogene*, vol. 8, no. 4, pp. 925–931, 1993.

[32] Y. Cao, W.-R. Ji, P. Qi, Å. Rosin, and Y. Cao, "Placenta growth factor: Identification and characterization of a novel isoform generated by RNA alternative splicing," *Biochemical and Biophysical Research Communications*, vol. 235, no. 3, pp. 493–498, 1997.

[33] J. E. Park, H. H. Chen, J. Winer, K. A. Houck, and N. Ferrara, "Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR," *The Journal of Biological Chemistry*, vol. 269, no. 41, pp. 25646–25654, 1994.

[34] A. Ahmed, X. F. Li, C. Dunk, M. J. Whittle, D. I. Rushton, and T. Rollason, "Colocalisation of vascular endothelial growth factor and its flt-1 receptor in human placenta," *Growth Factors*, vol. 12, no. 3, pp. 235–243, 1995.

[35] D. E. Clark, S. K. Smith, A. M. Sharkey, and D. S. Charnock-Jones, "Localization of VEGF and expression of its receptors flt and KDR in human placenta throughout pregnancy," *Human Reproduction*, vol. 11, no. 5, pp. 1090–1098, 1996.

[36] D. E. Clark, S. K. Smith, D. Licence, A. L. Evans, and D. S. Charnock-Jones, "Comparison of expression patterns for placenta growth factor, vascular endothelial growth factor (VEGF), VEGF-B and VEGF-C in the human placenta throughout gestation," *Journal of Endocrinology*, vol. 159, no. 3, pp. 459–467, 1998.

[37] A. Khaliq, X. F. Li, M. Shams et al., "Localisation of placenta growth factor (PIGF) in human term placenta," *Growth Factors*, vol. 13, no. 3-4, pp. 243–250, 1996.

[38] M. G. Persico, V. Vincenti, and T. DiPalma, "Structure, Expression and Receptor-Binding Properties of Placenta Growth Factor (PlGF)," in *Vascular Growth Factors and Angiogenesis*, vol. 237 of *Current Topics in Microbiology and Immunology*, pp. 31–40, Springer Berlin Heidelberg, Berlin, Heidelberg, 1999.

[39] G. Viglietto, D. Maglione, M. Rambaldi et al., "Upregulation of vascular endothelial growth factor (VEGF) and downregulation of placenta growth factor (P1GF) associated with malignancy in human thyroid tumors and cell lines," *Oncogene*, vol. 11, no. 8, pp. 1569–1579, 1995.

[40] G. Voros, E. Maquoi, D. Demeulemeester, N. Clerx, D. Collen, and H. R. Lijnen, "Modulation of angiogenesis during adipose

tissue development in murine models of obesity," *Endocrinology*, vol. 146, no. 10, pp. 4545–4554, 2005.

[41] C. De Vries, J. A. Escobedo, H. Ueno, K. Houck, N. Ferrara, and L. T. Williams, "The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor," *Science*, vol. 255, no. 5047, pp. 989–991, 1992.

[42] B. I. Terman, M. Dougher-Vermazen, M. E. Carrion et al., "Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor," *Biochemical and Biophysical Research Communications*, vol. 187, no. 3, pp. 1579–1586, 1992.

[43] M. Shibuya, S. Yamaguchi, A. Yamane et al., "Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely relatd to the fms family," *Oncogene*, vol. 5, no. 4, pp. 519–524, 1990.

[44] B. I. Terman, M. E. Carrion, E. Kovacs, B. A. Rasmussen, R. L. Eddy, and T. B. Shows, "Identification of a new endothelial cell growth factor receptor tyrosine kinase," *Oncogene*, vol. 6, no. 9, pp. 1677–1683, 1991.

[45] R. L. Kendall and K. A. Thomas, "Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 90, no. 22, pp. 10705–10709, 1993.

[46] R. J. Levine and S. A. Karumanchi, "Circulating angiogenic factors in preeclampsia," *Clinical Obstetrics and Gynecology*, vol. 48, no. 2, pp. 372–386, 2005.

[47] S. E. Maynard, J. Y. Min, J. Merchan et al., "Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction hypertension, and proteinuria in preeclampsia," *The Journal of Clinical Investigation*, vol. 111, no. 5, pp. 649–658, 2003.

[48] C. J. Robinson, D. D. Johnson, E. Y. Chang, D. M. Armstrong, and W. Wang, "Evaluation of placenta growth factor and soluble Fms-like tyrosine kinase 1 receptor levels in mild and severe preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 195, no. 1, pp. 255–259, 2006.

[49] A. C. Staff, K. Braekke, N. K. Harsem, T. Lyberg, and M. R. Holthe, "Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during preeclampsia," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 122, no. 1, pp. 33–39, 2005.

[50] V. Tsatsaris, F. Goffin, C. Munaut et al., "Overexpression of the Soluble Vascular Endothelial Growth Factor Receptor in Preeclamptic Patients: Pathophysiological Consequences," *The Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 11, pp. 5555–5563, 2003.

[51] K.-A. Wathén, E. Tuutti, U.-H. Stenman et al., "Maternal serum-soluble vascular endothelial growth factor receptor-1 in early pregnancy ending in preeclampsia or intrauterine growth retardation," *The Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 1, pp. 180–184, 2006.

[52] N. A. Bersinger and R. A. Ødegård, "Second- and third-trimester serum levels of placental proteins in preeclampsia and small-for-gestational age pregnancies," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 83, no. 1, pp. 37–45, 2004.

[53] R. J. Levine, S. E. Maynard, C. Qian et al., "Circulating angiogenic factors and the risk of preeclampsia," *The New England Journal of Medicine*, vol. 350, no. 7, pp. 672–683, 2004.

[54] T. Chaiworapongsa, R. Romero, J. Espinoza et al., "Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia: Young Investigator Award," *American Journal of Obstetrics & Gynecology*, vol. 190, no. 6, pp. 1541–1550, 2004.

[55] J.-Y. Chung, Y. Song, Y. Wang, R. R. Magness, and J. Zheng, "Differential Expression of Vascular Endothelial Growth Factor (VEGF), Endocrine Gland Derived-VEGF, and VEGF Receptors in Human Placentas from Normal and Preeclamptic Pregnancies," *The Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 5, pp. 2484–2490, 2004.

[56] A.-K. Wikström, A. Larsson, U. J. Eriksson, P. Nash, S. Nordén-Lindeberg, and M. Olovsson, "Placental growth factor and soluble FMS-like tyrosine kinase-1 in early-onset and late-onset preeclampsia," *Obstetrics & Gynecology*, vol. 109, no. 6, pp. 1368–1374, 2007.

[57] T. Chaiworapongsa, R. Romero, Y. M. Kim et al., "Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia," *The Journal of Maternal-Fetal and Neonatal Medicine*, vol. 17, no. 1, pp. 3–18, 2005.

[58] R. Romero, J. K. Nien, J. Espinoza et al., "A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate," *The Journal of Maternal-Fetal & Neonatal Medicine*, vol. 21, no. 1, pp. 9–23, 2009.

[59] M. Simon, H.-J. Grone, O. Johren et al., "Expression of vascular endothelial growth factor and its receptors in human renal ontogenesis and in adult kidney," *American Journal of Physiology - Renal Fluid and Electrolyte Physiology*, vol. 268, no. 2, pp. F240–F250, 1995.

[60] M. Simon, W. Röckl, C. Hornig et al., "Receptors of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) in fetal and adult human kidney: Localization and [125I]VEGF binding sites," *Journal of the American Society of Nephrology*, vol. 9, no. 6, pp. 1032–1044, 1998.

[61] S. Rana, C. E. Powe, S. Salahuddin et al., "Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia," *Circulation*, vol. 125, no. 7, pp. 911–919, 2012.

[62] R. Thadhani, W. P. Mutter, M. Wolf et al., "First trimester placental growth factor and soluble fms -like tyrosine kinase 1 and risk for preeclampsia," *The Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 2, pp. 770–775, 2004.

[63] B. M. Polliotti, A. G. Fry, D. N. Saller Jr., R. A. Mooney, C. Cox, and R. K. Miller, "Second-trimester maternal serum placental growth factor and vascular endothelial growth factor for predicting severe, early-onset preeclampsia," *Obstetrics & Gynecology*, vol. 101, no. 6, pp. 1266–1274, 2003.

[64] L. C. Y. Poon, N. A. Kametas, N. Maiz, R. Akolekar, and K. H. Nicolaides, "First-trimester prediction of hypertensive disorders in pregnancy," *Hypertension*, vol. 53, no. 5, pp. 812–818, 2009.

[65] Y. N. Su, C. N. Lee, W. F. Cheng, W. Y. Shau, S. N. Chow, and F. J. Hsieh, "Decreased maternal serum placenta growth factor in early second trimester and preeclampsia," *Obstetrics & Gynecology*, vol. 97, no. 6, pp. 898–904, 2001.

[66] L. J. Vatten, A. Eskild, T. I. L. Nilsen, S. Jeansson, P. A. Jenum, and A. C. Staff, "Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 196, no. 3, pp. 239–e6, 2007.

[67] D. S. Torry, H.-S. Wang, T.-H. Wang, M. R. Caudle, and R. J. Torry, "Preeclampsia is associated with reduced serum levels of placenta growth factor," *American Journal of Obstetrics & Gynecology*, vol. 179, no. 6 I, pp. 1539–1544, 1998.

[68] R. Akolekar, E. Zaragoza, L. C. Y. Poon, S. Pepes, and K. H. Nicolaides, "Maternal serum placental growth factor at 11 + 0 to 13 + 6 weeks of gestation in the prediction of pre-eclampsia," *Ultrasound in Obstetrics & Gynecology*, vol. 32, pp. 732–773, 2009.

[69] T. Krauss, H. Pauer, and H. G. Augustin, "Prospective analysis of placenta growth factor (PlGF) concentrations in the plasma of women with normal pregnancy and pregnancies complicated by preeclampsia," *Hypertension in Pregnancy*, vol. 23, no. 1, pp. 101–111, 2004.

[70] R. N. Taylor, J. Grimwood, R. S. Taylor, M. T. McMaster, S. J. Fisher, and R. A. North, "Longitudinal serum concentrations of placental growth factor: Evidence for abnormal placental angiogenesis in pathologic pregnancies," *American Journal of Obstetrics & Gynecology*, vol. 188, no. 1, pp. 177–182, 2003.

[71] S. C. Tidwell, H. Ho, W. Chiu, R. J. Torry, and D. S. Torry, "Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 184, no. 6, pp. 1267–1272, 2001.

[72] M. L. Tjoa, J. M. G. van Vugt, M. A. M. Mulders, R. B. H. Schutgens, C. B. M. Oudejans, and I. J. van Wijk, "Plasma placenta growth factor levels in midtrimester pregnancies," *Obstetrics & Gynecology*, vol. 98, no. 4, pp. 600–607, 2001.

[73] D. P. Holden, S. A. Fickling, G. S. Whitley, and S. S. Nussey, "Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 178, no. 3, pp. 551–556, 1998.

[74] Y. J. Kim, H. S. Park, H. Y. Lee et al., "Reduced L-arginine level and decreased placental eNOS activity in preeclampsia," *Placenta*, vol. 27, no. 4-5, pp. 438–444, 2006.

[75] M. Laskowska and J. Oleszczuk, "Evaluation of ADMA levels in women with pregnancies complicated by severe preeclampsia," *Archives of Perinatal Medicine*, vol. 17, no. 1, pp. 33–36, 2011.

[76] R. Maas, R. H. Böger, E. Schwedhelm et al., "Plasma Concentrations of Asymmetric Dimethylarginine (ADMA) in Colombian Women with Pre-eclampsia [4]," *Journal of the American Medical Association*, vol. 291, no. 7, pp. 823-824, 2004.

[77] M. Noorbakhsh, M. Kianpour, and M. Nematbakhsh, "Serum Levels of Asymmetric Dimethylarginine, Vascular Endothelial Growth Factor, and Nitric Oxide Metabolite Levels in Preeclampsia Patients," *ISRN Obstetrics and Gynecology*, vol. 2013, pp. 1–5, 2013.

[78] E. Gumus, M. A. Atalay, B. Cetinkaya Demir, and E. Sahin Gunes, "Possible role of asymmetric dimethylarginine (ADMA) in prediction of perinatal outcome in preeclampsia and fetal growth retardation related to preeclampsia," *The Journal of Maternal-Fetal and Neonatal Medicine*, vol. 29, no. 23, pp. 3806–3811, 2016.

[79] T. Maeda, T. Yoshimura, and H. Okamura, "Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, in maternal and fetal circulation," *J. Soc. Gynecol. Investig*, vol. 10, p. 24, 2003.

[80] A. Pettersson, T. Hedner, and I. Milsom, "Increased circulating concentrations of asymmetric dimethyl arginine (ADMA, an endogenous inhibitor of nitric oxide synthesis, in preeclampsia," *Acta Obstet. Gynecol. Scand*, vol. 77, pp. 808–813, 1998.

[81] D. Rizos, M. Eleftheriades, E. Batakis et al., "Levels of asymmetric dimethylarginine throughout normal pregnancy and in pregnancies complicated with preeclampsia or had a small for gestational age baby," *The Journal of Maternal-Fetal and Neonatal Medicine*, vol. 25, no. 8, pp. 1311–1315, 2012.

[82] P. D. Speer, R. W. Powers, M. P. Frank, G. Harger, N. Markovic, and J. M. Roberts, "Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational-age infants," *American Journal of Obstetrics & Gynecology*, vol. 198, no. 1, pp. 112.e1–112.e7, 2008.

[83] M. D. Savvidou, A. D. Hingorani, D. Tsikas, J. C. Frölich, P. Vallance, and K. H. Nicolaides, "Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia," *The Lancet*, vol. 361, no. 9368, pp. 1511–1517, 2003.

[84] H. Tsukahara, N. Ohta, S. Tokuriki et al., "Determination of asymmetric dimethylarginine, an endogenous nitric oxide synthase inhibitor, in umbilical blood," *Metabolism - Clinical and Experimental*, vol. 57, no. 2, pp. 215–220, 2008.

[85] A. Jeyabalan, S. McGonigal, C. Gilmour, C. A. Hubel, and A. Rajakumar, "Circulating and placental endoglin concentrations in pregnancies complicated by intrauterine growth restriction and preeclampsia," *Placenta*, vol. 29, no. 6, pp. 555–563, 2008.

[86] C. J. Robinson and D. D. Johnson, "Soluble endoglin as a second-trimester marker for preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 197, no. 2, pp. 174.e1–174.e5, 2007.

[87] R. J. Levine, C. Lam, C. Qian et al., "Soluble endoglin and other circulating antiangiogenic factors in preeclampsia," *The New England Journal of Medicine*, vol. 355, no. 10, pp. 992–1005, 2006.

[88] S. Rana, S. A. Karumanchi, R. J. Levine et al., "Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia," *Hypertension*, vol. 50, no. 1, pp. 137–142, 2007.

[89] H. Stepan, T. Krämer, and R. Faber, "Maternal plasma concentrations of soluble endoglin in pregnancies with intrauterine growth restriction," *The Journal of Clinical Endocrinology & Metabolism*, vol. 92, no. 7, pp. 2831–2834, 2007.

[90] H. Stepan, A. Geipel, F. Schwarz, T. Krämer, N. Wessel, and R. Faber, "Circulatory soluble endoglin and its predictive value for preeclampsia in second-trimester pregnancies with abnormal uterine perfusion," *American Journal of Obstetrics & Gynecology*, vol. 198, no. 2, pp. 175–e6, 2008.

[91] S. Venkatesha, M. Toporsian, C. Lam et al., "Erratum: Soluble endoglin contributes to the pathogenesis of preeclampsia (Nature Medicine (2006) 12, (642-649))," *Nature Medicine*, vol. 12, no. 7, p. 862, 2006.

[92] O. Burger, E. Pick, J. Zwickel et al., "Placental protein 13 (PP-13): Effects on cultured trophoblasts, and its detection in human body fluids in normal and pathological pregnancies," *Placenta*, vol. 25, no. 7, pp. 608–622, 2004.

[93] B. Huppertz, M. Sammar, I. Chefetz, P. Neumaier-Wagner, C. Bartz, and H. Meiri, "Longitudinal determination of serum placental protein 13 during development of preeclampsia," *Fetal Diagnosis and Therapy*, vol. 24, no. 3, pp. 230–236, 2008.

[94] I. Chafetz, I. Kuhnreich, M. Sammar et al., "First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction," *American Journal of Obstetrics & Gynecology*, vol. 197, no. 1, pp. 35.e1–35.e7, 2007.

[95] A. Khalil, N. J. Cowans, K. Spencer, S. Goichman, H. Meiri, and K. Harrington, "First trimester maternal serum placental protein 13 for the prediction of pre-eclampsia in women with a

priori high risk," *Prenatal Diagnosis*, vol. 29, no. 8, pp. 781–789, 2009.

[96] K. H. Nicolaides, R. Bindra, O. M. Turan et al., "A novel approach to first-trimester screening for early pre-eclampsia combining serum PP-13 and Doppler ultrasound," *Ultrasound in Obstetrics & Gynecology*, vol. 27, no. 1, pp. 13–17, 2006.

[97] K. Spencer, N. J. Cowans, I. Chefetz, J. Tal, I. Kuhnreich, and H. Meiri, "Second-trimester uterine artery Doppler pulsatility index and maternal serum PP13 as markers of pre-eclampsia," *Prenatal Diagnosis*, vol. 27, no. 3, pp. 258–263, 2007.

[98] K. Spencer, N. J. Cowans, I. Chefetz, J. Tal, and H. Meiri, "First-trimester maternal serum PP-13, PAPP-A and second-trimester uterine artery Doppler pulsatility index as markers of pre-eclampsia," *Ultrasound in Obstetrics & Gynecology*, vol. 29, no. 2, pp. 128–134, 2007.

[99] R. Romero, J. P. Kusanovic, N. G. Than et al., "First-trimester maternal serum PP13 in the risk assessment for preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 199, no. 2, pp. 122–e11, 2008.

[100] H. Aksoy, Y. Kumtepe, F. Akçay, and A. K. Yildirim, "Correlation of P-selectin and lipoprotein(a), and other lipid parameters in preeclampsia," *Clinical and Experimental Medicine*, vol. 2, no. 1, pp. 39–43, 2002.

[101] I. Banzola, A. Farina, M. Concu et al., "Performance of a panel of maternal serum markers in predicting preeclampsia at 11-15 weeks' gestation," *Prenatal Diagnosis*, vol. 27, no. 11, pp. 1005–1010, 2007.

[102] F. Bretelle, F. Sabatier, D. Desprez et al., "Circulating microparticles: a marker of procoagulant state in normal pregnancy and pregnancy complicated by preeclampsia or intrauterine growth restriction," *Thrombosis and Haemostasis*, vol. 89, no. 3, pp. 486–492, 2003.

[103] A. Halim, N. Kanayama, E. El Maradny et al., "Plasma P selectin (GMP-140) and glycocalicin are elevated in preeclampsia and eclampsia: Their significances," *American Journal of Obstetrics & Gynecology*, vol. 174, no. 1, pp. 272–277, 1996.

[104] W. Heyl, S. Handt, F. Reister et al., "Elevated soluble adhesion molecules in women with pre-eclampsia," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 86, no. 1, pp. 35–41, 1999.

[105] C. A. R. Lok, R. Nieuwland, A. Sturk et al., "Microparticle-associated P-selectin reflects platelet activation in preeclampsia," *Platelets*, vol. 18, no. 1, pp. 68–72, 2007.

[106] T. Chaiworapongsa, R. Romero, J. Yoshimatsu et al., "Soluble adhesion molecule profile in normal pregnancy and pre-eclampsia," *The Journal of Maternal-Fetal & Neonatal Medicine*, vol. 12, no. 1, pp. 19–27, 2009.

[107] P. Bosio, S. Cannon, P. McKenna, C. O'Herlihy, R. Conroy, and H. Brady, "Plasma P-selectin is elevated in the first trimester in women who subsequently develop pre-eclampsia," *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 108, no. 7, pp. 709–715, 2001.

[108] M. E. Chavarría, L. Lara-González, Y. García-Paleta, V. S. Vital-Reyes, and A. Reyes, "Adhesion molecules changes at 20 gestation weeks in pregnancies complicated by preeclampsia," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 137, no. 2, pp. 157–164, 2008.

[109] A. A. Senna, M. Zedan, G. E. Abd El Salam, and A. I. El Mashad, "Study of plasma adrenomedullin level in normal pregnancy and preclampsia," *The Medscape Journal of Medicine*, vol. 10, no. 2, article no. 29, 2008.

[110] J. Laigaard, T. Sørensen, S. Placing et al., "Reduction of the disintegrin and metalloprotease ADAM12 in preeclampsia," *Obstetrics & Gynecology*, vol. 106, no. 1, pp. 144–149, 2005.

[111] K. H. Nicolaides, L. C. Y. Poon, T. Chelemen, O. Granvillano, and I. Pandeva, "First-trimester maternal serum a disintegrin and metalloprotease 12 (ADAM12) and adverse pregnancy outcome," *Obstetrics & Gynecology*, vol. 112, no. 5, pp. 1082–1090, 2008.

[112] K. Spencer, N. J. Cowans, and A. Stamatopoulou, "ADAM12s in maternal serum as a potential marker of pre-eclampsia," *Prenatal Diagnosis*, vol. 28, no. 3, pp. 212–216, 2008.

[113] I. Cetin, V. Cozzi, F. Pasqualini et al., "Elevated maternal levels of the long pentraxin 3 (PTX3) in preeclampsia and intrauterine growth restriction," *American Journal of Obstetrics & Gynecology*, vol. 194, no. 5, pp. 1347–1353, 2006.

[114] P. Rovere-Querini, S. Antonacci, G. Dell'Antonio et al., "Plasma and tissue expression of the long pentraxin 3 during normal pregnancy and preeclampsia," *Obstetrics & Gynecology*, vol. 108, no. 1, pp. 148–155, 2006.

[115] N. J. Cowans and K. Spencer, "First-trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system," *Prenatal Diagnosis*, vol. 27, no. 3, pp. 264–271, 2007.

[116] C. Y. T. Ong, A. W. Liao, K. Spencer, S. Munim, and K. H. Nicolaides, "First trimester maternal serum free β human chorionic gonadotrophin and pregnancy associated plasma protein a as predictors of pregnancy complications," *British Journal of Obstetrics and Gynaecology*, vol. 107, no. 10, pp. 1265–1270, 2000.

[117] K. Spencer, N. J. Cowans, and K. H. Nicolaides, "Low levels of maternal serum PAPP-A in the first trimester and the risk of pre-eclampsia," *Prenatal Diagnosis*, vol. 28, no. 1, pp. 7–10, 2008.

[118] Y. Yaron, S. Heifetz, Y. Ochshorn, O. Lehavi, and A. Orr-Urtreger, "Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome," *Prenatal Diagnosis*, vol. 22, no. 9, pp. 778–782, 2002.

[119] L. Dugoff, J. C. Hobbins, F. D. Malone et al., "First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial)," *American Journal of Obstetrics & Gynecology*, vol. 191, no. 4, pp. 1446–1451, 2004.

[120] D. Krantz, L. Goetzl, J. L. Simpson et al., "Association of extreme first-trimester free human chorionic gonadotropin-β, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes," *American Journal of Obstetrics & Gynecology*, vol. 191, no. 4, pp. 1452–1458, 2004.

[121] K. Spencer, N. J. Cowans, F. Molina, K. O. Kagan, and K. H. Nicolaides, "First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of preterm or early preterm delivery," *Ultrasound in Obstetrics & Gynecology*, vol. 31, no. 2, pp. 147–152, 2008.

[122] N. Tul, S. Pusenjak, J. Osredkar, K. Spencer, and Z Novak-Antolic, "Predicting complications of pregnancy with first-trimester maternal serum free-betahCG, PAPP-A and inhibin-A," *Prenatal Diagnosis*, vol. 23, no. 12, pp. 990–996, 2003.

[123] G. C. Smith, E. J. Stenhouse, J. A. Crossley, D. A. Aitken, A. D. Cameron, and J. M. Connor, "Early Pregnancy Levels of Pregnancy-Associated Plasma Protein A and the Risk of

Intrauterine Growth Restriction, Premature Birth, Preeclampsia, and Stillbirth," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 4, pp. 1762–1767, 2002.

[124] W. Hu, Z. Wang, H. Wang, H. Huang, and M. Dong, "Serum visfatin levels in late pregnancy and pre-eclampsia," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 87, no. 4, pp. 413–418, 2008.

[125] A. Farina, A. Sekizawa, M. Iwasaki, R. Matsuoka, K. Ichizuka, and T. Okai, "Total cell-free DNA (β-globin gene) distribution in maternal plasma at the second trimester: A new prospective for preeclampsia screening," *Prenatal Diagnosis*, vol. 24, no. 9, pp. 722–726, 2004.

[126] A. Sekizawa, A. Farina, K. Koide et al., "β-globin DNA in maternal plasma as a molecular marker of pre-eclampsia," *Prenatal Diagnosis*, vol. 24, no. 9, pp. 697–700, 2004.

[127] D. W. Swinkels, J. B. De Kok, J. C. M. Hendriks, E. Wiegerinck, P. L. M. Zusterzeel, and E. A. P. Steegers, "Hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome as a complication of preeclampsia in pregnant women increases the amount of cell-free fetal and maternal DNA in maternal plasma and serum," *Clinical Chemistry*, vol. 48, no. 4, pp. 650–653, 2002.

[128] Y. M. D. Lo, M. S. C. Tein, T. K. Lau et al., "Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis," *American Journal of Human Genetics*, vol. 62, no. 4, pp. 768–775, 1998.

[129] X. Y. Zhong, H. Laivuori, J. C. Livingston et al., "Elevation of both maternal and fetal extracellular circulating deoxyribonucleic acid concentrations in the plasma of pregnant women with preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 184, no. 3, pp. 414–419, 2001.

[130] R. J. Levine, C. Qian, E. S. Leshane et al., "Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 190, no. 3, pp. 707–713, 2004.

[131] R. M. Silver, L. Myatt, J. C. Hauth et al., "Cell-Free Total and Fetal DNA in First Trimester Maternal Serum and Subsequent Development of Preeclampsia," *American Journal of Perinatology*, vol. 34, no. 2, pp. 191–198, 2017.

[132] X. Y. Zhong, W. Holzgreve, and S. Hahn, "The levels of circulatory cell free fetal DNA in maternal plasma are elevated prior to the onset of preeclampsia," *Hypertension in Pregnancy*, vol. 21, no. 1, pp. 77–83, 2002.

[133] N. Leung Tse, Jun. Zhang, K. Tze, Y. S. Lisa, and Y. M. Dennis Lo, *Increased Maternal Plasma Fetal DNA Concentrations in Women Who Eventually Develop Preeclampsia. Clinical Chemistry*, vol. 47, 'Increased Maternal Plasma Fetal DNA Concentrations in Women Who Eventually Develop Preeclampsia.' Clinical Chemistry 47 (1, 137–39, 2001.

[134] S. De Falco, "The discovery of placenta growth factor and its biological activity," *Experimental & Molecular Medicine*, vol. 44, no. 1, p. 1, 2012.

[135] P. Vuorela, E. Hatva, A. Lymboussaki et al., "Expression of vascular endothelial growth factor and placenta growth factor in human placenta," *Biology of Reproduction*, vol. 56, no. 2, pp. 489–494, 1997.

[136] J. Dommisse and A. J. Tiltman, "Placental bed biopsies in placental abruption," *An International Journal of Obstetrics & Gynaecology*, vol. 99, no. 8, pp. 651–654, 1992.

[137] T. K. Eskes, "Abruptio placentae," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 75, no. 1, pp. 63–70, 1997.

[138] R. Thadhani, J. L. Ecker, W. P. Mutter et al., "Insulin Resistance and Alterations in Angiogenesis: Additive Insults That May Lead to Preeclampsia," *Hypertension*, vol. 43, no. 5, pp. 988–992, 2004.

[139] H. Stepan, A. Unversucht, N. Wessel, and R. Faber, "Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion," *Hypertension*, vol. 49, no. 4, pp. 818–824, 2007.

[140] C. S. Buhimschi, E. R. Norwitz, E. Funai et al., "Urinary angiogenic factors cluster hypertensive disorders and identify women with severe preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 192, no. 3, pp. 734–741, 2005.

[141] A. C. Staff, K. Braekke, G. M. Johnsen, S. A. Karumanchi, and N. K. Harsem, "Circulating concentrations of soluble endoglin (CD105) in fetal and maternal serum and in amniotic fluid in preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 197, no. 2, pp. 176–e6, 2007.

[142] B. C. Smith and J. M. Denu, "Chemical mechanisms of histone lysine and arginine modifications," *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, vol. 1789, no. 1, pp. 45–57, 2009.

[143] A. Scoumanne and X. Chen, "Protein methylation: A new mechanism of p53 tumor suppressor regulation," *Histology and Histopathology*, vol. 23, no. 9, pp. 1143–1149, 2008.

[144] M. T. Bedford and S. Richard, "Arginine methylation: an emerging regulator of protein function," *Molecular Cell*, vol. 18, no. 3, pp. 263–272, 2005.

[145] L. Sibal, S. C. Agarwal, P. D. Home, and R. H. Boger, "The role of asymmetric dimethylarginine (ADMA) in endothelial dysfunction and cardiovascular disease," *Current Cardiology Reviews*, vol. 6, no. 2, pp. 82–90, 2010.

[146] S. G. Clarke, "Protein methylation at the surface and buried deep: Thinking outside the histone box," *Trends in Biochemical Sciences*, vol. 38, no. 5, pp. 243–252, 2013.

[147] T. Ogawa, M. Kimoto, and K. Sasaoka, "Occurrence of a new enzyme catalyzing the direct conversion of NG,NG-dimethyl-L-arginine to L-citrulline in rats," *Biochemical and Biophysical Research Communications*, vol. 148, no. 2, pp. 671–677, 1987.

[148] A. Ito, P. S. Tsao, S. Adimoolam, M. Kimoto, T. Ogawa, and J. P. Cooke, "Novel mechanism for endothelial dysfunction: Dysregulation of dimethylarginine dimethylaminohydrolase," *Circulation*, vol. 99, no. 24, pp. 3092–3095, 1999.

[149] P. Vallance, A. Leone, A. Calver, J. Collier, and S. Moncada, "Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure," *The Lancet*, vol. 339, no. 8793, pp. 572–575, 1992.

[150] M. S. Goligorsky, "Endothelial cell dysfunction: can't live with it, how to live without it," *American Journal of Physiology-Renal Physiology*, vol. 288, no. 5, pp. F871–F880, 2005.

[151] J. P. Cooke, E. Rossitch Jr., N. A. Andon, J. Loscalzo, and V. J. Dzau, "Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator," *The Journal of Clinical Investigation*, vol. 88, no. 5, pp. 1663–1671, 1991.

[152] R. F. Furchgott and J. V. Zawadzki, "The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine," *Nature*, vol. 288, no. 5789, pp. 373–376, 1980.

[153] A. Wolf, C. Zalpour, G. Theilmeier et al., "Dietary L-arginine supplementation normalizes platelet aggregation in hypercholesterolemic humans," *Journal of the American College of Cardiology*, vol. 29, no. 3, pp. 479–485, 1997.

[154] P. Kubes, M. Suzuki, and D. N. Granger, "Nitric oxide: an endogenous modulator of leukocyte adhesion," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 88, no. 11, pp. 4651–4655, 1991.

[155] R. H. Böger, "Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor," *Journal of Nutrition*, vol. 134, no. 10, pp. 2842S–2847S, 2004.

[156] R. J. MacAllister, H. Parry, M. Kimoto et al., "Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase," *British Journal of Pharmacology*, vol. 119, no. 8, pp. 1533–1540, 1996.

[157] M. C. Stühlinger, R. K. Oka, E. E. Graf et al., "Endothelial Dysfunction Induced by Hyperhomocyst(e)inemia: Role of Asymmetric Dimethylarginine," *Circulation*, vol. 108, pp. 933–938, 2003.

[158] K. Y. Lin, A. Ito, T. Asagami et al., "Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase," *Circulation*, vol. 106, no. 8, pp. 987–992, 2002.

[159] M. Weis, T. N. Kledal, K. Y. Lin et al., "Cytomegalovirus Infection Impairs the Nitric Oxide Synthase Pathway: Role of Asymmetric Dimethylarginine in Transplant Arteriosclerosis," *Circulation*, vol. 109, no. 4, pp. 500–505, 2004.

[160] M. C. Stühlinger, P. S. Tsao, J.-H. Her, M. Kimoto, R. F. Balint, and J. P. Cooke, "Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine," *Circulation*, vol. 104, no. 21, pp. 2569–2575, 2001.

[161] J.-F. Couture, L. M. A. Dirk, J. S. Brunzelle, R. L. Houtz, and R. C. Trievel, "Structural origins for the product specificity of SET domain protein methyltransferases," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 105, no. 52, pp. 20659–20664, 2008.

[162] X. Zhang and T. C. Bruice, "Enzymatic mechanism and product specificity of SET-domain protein lysine methyltransferases," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 105, no. 15, pp. 5728–5732, 2008.

[163] M. T. Bedford and S. G. Clarke, "Protein arginine methylation in mammals: who, what, and why," *Molecular Cell*, vol. 33, no. 1, pp. 1–13, 2009.

[164] Y. Yang and M. T. Bedford, "Protein arginine methyltransferases and cancer," *Nature Reviews Cancer*, vol. 13, no. 1, pp. 37–50, 2013.

[165] C. I. Zurita-Lopez, T. Sandberg, R. Kelly, and S. G. Clarke, "Human protein arginine methyltransferase 7 (PRMT7) is a type III enzyme forming ω-NG-monomethylated arginine residues," *The Journal of Biological Chemistry*, vol. 287, no. 11, pp. 7859–7870, 2012.

[166] H. L. Schubert, R. M. Blumenthal, and X. Cheng, "Many paths to methyltransfer: A chronicle of convergence," *Trends in Biochemical Sciences*, vol. 28, no. 6, pp. 329–335, 2003.

[167] P. A. del Rizzo and R. C. Trievel, "Substrate and product specificities of SET domain methyltransferases," *Epigenetics*, vol. 6, no. 9, pp. 1059–1067, 2011.

[168] K. L. Tkaczuk, S. Dunin-Horkawicz, E. Purta, and J. M. Bujnicki, "Structural and evolutionary bioinformatics of the SPOUT superfamily of methyltransferases," *BMC Bioinformatics*, vol. 8, article 73, 2007.

[169] Q. Feng, H. Wang, H. H. Ng et al., "Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain," *Current Biology*, vol. 12, no. 12, pp. 1052–1058, 2002.

[170] C. Qian and M.-M. Zhou, "SET domain protein lysine methyltransferases: structure, specificity and catalysis," *Cellular and Molecular Life Sciences*, vol. 63, no. 23, pp. 2755–2763, 2006.

[171] T. C. Petrossian and S. G. Clarke, "Uncovering the human methyltransferasome," *Molecular & Cellular Proteomics*, vol. 10, no. 1, 2011.

[172] V. M. Richon, D. Johnston, C. J. Sneeringer et al., "Chemogenetic Analysis of Human Protein Methyltransferases," *Chemical Biology & Drug Design*, vol. 78, no. 2, pp. 199–210, 2011.

[173] S. Kernstock, E. Davydova, M. Jakobsson et al., "Lysine methylation of VCP by a member of a novel human protein methyltransferase family," *Nature Communications*, vol. 3, article no. 1038, 2012.

[174] R. Magnani, L. M. A. Dirk, R. C. Trievel, and R. L. Houtz, "Calmodulin methyltransferase is an evolutionarily conserved enzyme that trimethylates Lys-115 in calmodulin," *Nature Communications*, vol. 1, no. 4, 2010.

[175] A. T. Nguyen and Y. Zhang, "The diverse functions of Dot1 and H3K79 methylationanh," *Genes & Development*, vol. 25, no. 13, pp. 1345–1358, 2011.

[176] K. J. Webb, Q. Al-Hadid, C. I. Zurita-Lopez, B. D. Young, R. S. Lipson, and S. G. Clarke, "The ribosomal L1 protuberance in yeast is methylated on a lysine residue catalyzed by a seven-β-strand methyltransferase," *The Journal of Biological Chemistry*, vol. 286, no. 21, pp. 18405–18413, 2011.

[177] S. C. Dillon, X. Zhang, R. C. Trievel, and X. Cheng, "The SET-domain protein superfamily: Protein lysine methyltransferases," *Genome Biology*, vol. 6, no. 8, article no. 227, 2005.

[178] T. Shimazu, J. Barjau, Y. Sohtome, M. Sodeoka, and Y. Shinkai, "Selenium-based S-adenosylmethionine analog reveals the mammalian seven-beta-strand methyltransferase METTL10 to be an EF1A1 lysine methyltransferase," *PLoS ONE*, vol. 9, no. 8, Article ID e105394, 2014.

[179] P. Cloutier, M. Lavallée-Adam, D. Faubert, M. Blanchette, and B. Coulombe, "A Newly Uncovered Group of Distantly Related Lysine Methyltransferases Preferentially Interact with Molecular Chaperones to Regulate Their Activity," *PLoS Genetics*, vol. 9, no. 1, Article ID e1003210, 2013.

[180] J. C. Black, C. Van Rechem, and J. R. Whetstine, "Histone lysine methylation dynamics: establishment, regulation, and biological impact," *Molecular Cell*, vol. 48, no. 4, pp. 491–507, 2012.

[181] J. C. Eissenberg and A. Shilatifard, "Histone H3 lysine 4 (H3K4) methylation in development and differentiation," *Developmental Biology*, vol. 339, no. 2, pp. 240–249, 2010.

[182] R. A. Greenberg, "Histone tails: Directing the chromatin response to DNA damage," *FEBS Letters*, vol. 585, no. 18, pp. 2883–2890, 2011.

[183] T. Kouzarides, "Chromatin modifications and their function," *Cell*, vol. 128, no. 4, pp. 693–705, 2007.

[184] A. Nottke, M. P. Colaiácovo, and Y. Shi, "Developmental roles of the histone lysine demethylases," *Development*, vol. 136, no. 6, pp. 879–889, 2009.

[185] M. T. Pedersen and K. Helin, "Histone demethylases in development and disease," *Trends in Cell Biology*, vol. 20, no. 11, pp. 662–671, 2010.

[186] J. C. Black and J. R. Whetstine, "Tipping the lysine methylation balance in disease," *Biopolymers*, vol. 99, no. 2, pp. 127–135, 2013.

[187] E. L. Greer and Y. Shi, "Histone methylation: a dynamic mark in health, disease and inheritance," *Nature Reviews Genetics*, vol. 13, no. 5, pp. 343–357, 2012.

[188] R. A. Copeland, M. P. Moyer, and V. M. Richon, "Targeting genetic alterations in protein methyltransferases for personalized cancer therapeutics," *Oncogene*, vol. 32, no. 8, pp. 939–946, 2013.

[189] S. Hake, A. Xiao, and C. Allis, "Linking the epigenetic 'language' of covalent histone modifications to cancer," *British Journal of Cancer*, vol. 90, no. 4, pp. 761–769, 2004.

[190] H. Ü. Kaniskan and J. Jin, "Chemical probes of histone lysine methyltransferases," *ACS Chemical Biology*, vol. 10, no. 1, pp. 40–50, 2015.

[191] R. A. Varier and H. T. M. Timmers, "Histone lysine methylation and demethylation pathways in cancer," *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, vol. 1815, no. 1, pp. 75–89, 2011.

[192] E. A. Pollina and A. Brunet, "Epigenetic regulation of aging stem cells," *Oncogene*, vol. 30, no. 28, pp. 3105–3126, 2011.

[193] M. Albert and K. Helin, "Histone methyltransferases in cancer," *Seminars in Cell & Developmental Biology*, vol. 21, no. 2, pp. 209–220, 2010.

[194] P. Chi, C. D. Allis, and G. G. Wang, "Covalent histone modifications-miswritten, misinterpreted and mis-erased in human cancers," *Nature Reviews Cancer*, vol. 10, no. 7, pp. 457–469, 2010.

[195] S. Varambally, S. M. Dhanasekaran, M. Zhou et al., "The polycomb group protein EZH2 is involved in progression of prostate cancer," *Nature*, vol. 419, no. 6907, pp. 624–629, 2002.

[196] C. G. Kleer, Q. Cao, S. Varambally et al., "EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 100, no. 20, pp. 11606–11611, 2003.

[197] H. P. J. Visser, M. J. Gunster, H. C. Kluin-Nelemans et al., "The Polycomb group protein EZH2 is upregulated in proliferating, cultured human mantle cell lymphoma," *British Journal of Haematology*, vol. 112, no. 4, pp. 950–958, 2001.

[198] R. Chen, Y. Tan, M. Wang et al., "Development of glycoprotein capture-based label-free method for the high-throughput screening of differential glycoproteins in hepatocellular carcinoma," *Molecular ##hssm###38; Cellular Proteomics*, vol. 10, no. 7, Article ID M110.006445, 2011.

[199] R. Hamamoto, V. Saloura, and Y. Nakamura, "Critical roles of non-histone protein lysine methylation in human tumorigenesis," *Nature Reviews Cancer*, vol. 15, no. 2, pp. 110–124, 2015.

[200] S. Ko, J. Ahn, C. S. Song, S. Kim, K. Knapczyk-Stwora, and B. Chatterjee, "Lysine methylation and functional modulation of androgen receptor by set9 methyltransferase," *Molecular Endocrinology*, vol. 25, no. 3, pp. 433–444, 2011.

[201] S. C. Smith, P. N. Baker, and E. M. Symonds, "Placental apoptosis in normal human pregnancy," *American Journal of Obstetrics & Gynecology*, vol. 177, no. 1, pp. 57–65, 1997.

[202] A. D. Allaire, K. A. Ballenger, S. R. Wells, M. J. McMahon, and B. A. Lessey, "Placental apoptosis in preeclampsia," *Obstetrics & Gynecology*, vol. 96, no. 2, pp. 271–276, 2000.

[203] A. E. P. Heazell, H. R. Buttle, P. N. Baker, and I. P. Crocker, "Altered expression of regulators of caspase activity within trophoblast of normal pregnancies and pregnancies complicated by preeclampsia," *Reproductive Sciences*, vol. 15, no. 10, pp. 1034–1043, 2008.

[204] D. N. Leung, S. C. Smith, K. To, D. S. Sahota, and P. N. Baker, "Increased placental apoptosis in pregnancies complicated by preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 184, no. 6, pp. 1249-1250, 2001.

[205] B. Huppertz and J. C. P. Kingdom, "Apoptosis in the trophoblast—role of apoptosis in placental morphogenesis," *Journal of the Society for Gynecologic Investigation*, vol. 11, no. 6, pp. 353–362, 2004.

[206] A. N. Sharp, A. E. Heazell, I. P. Crocker, and G. Mor, "Placental Apoptosis in Health and Disease," *American Journal of Reproductive Immunology*, vol. 64, no. 3, pp. 159–169, 2010.

[207] A. N. Sharp, A. E. P. Heazell, D. Baczyk et al., "Preeclampsia is associated with alterations in the p53-pathway in villous trophoblast," *PLoS ONE*, vol. 9, no. 1, Article ID e87621, 2014.

[208] T. Hung, S. Chen, L. Lo et al., "Increased Autophagy in Placentas of Intrauterine Growth-Restricted Pregnancies," *PLoS ONE*, vol. 7, no. 7, p. e40957, 2012.

[209] R. Levy, S. D. Smith, K. Yusuf et al., "Trophoblast apoptosis from pregnancies complicated by fetal growth restriction is associated with enhanced p53 expression," *American Journal of Obstetrics & Gynecology*, vol. 186, no. 5, pp. 1056–1061, 2002.

[210] S. Chuikov, J. K. Kurash, J. R. Wilson et al., "Regulation of p53 activity through lysine methylation," *Nature*, vol. 432, no. 7015, pp. 353–360, 2004.

[211] J. Oyake, M. Otaka, T. Matsuhashi et al., "Over-expression of 70-kDa heat shock protein confers protection against monochloramine-induced gastric mucosal cell injury," *Life Sciences*, vol. 79, no. 3, pp. 300–305, 2006.

[212] S. Jirecek, M. Hohlagschwandtner, C. Tempfer, M. Knöfler, P. Husslein, and H. Zeisler, "Serum levels of heat shock protein 70 in patients with preeclampsia: A pilot-study," *Wiener Klinische Wochenschrift*, vol. 114, no. 15-16, pp. 730–732, 2002.

[213] J. Hageman, M. A. W. H. van Waarde, A. Zylicz, D. Walerych, and H. H. Kampinga, "The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities," *Biochemical Journal*, vol. 435, no. 1, pp. 127–142, 2011.

[214] E. L. Davies, M. M. F. V. G. Bacelar, M. J. Marshall et al., "Heat shock proteins form part of a danger signal cascade in response to lipopolysaccharide and GroEL," *Clinical & Experimental Immunology*, vol. 145, no. 1, pp. 183–189, 2006.

[215] X. Luo, X. Zuo, B. Zhang et al., "Release of heat shock protein 70 and the effects of extracellular heat shock protein 70 on the production of IL-10 in fibroblast-like synoviocytes," *Cell Stress and Chaperones*, vol. 13, no. 3, pp. 365–373, 2008.

[216] D. G. Millar, K. M. Garza, B. Odermatt et al., "Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo," *Nature Medicine*, vol. 9, no. 12, pp. 1469–1476, 2003.

[217] H. H. Kampinga, J. Hageman, M. J. Vos et al., "Guidelines for the nomenclature of the human heat shock proteins," *Cell Stress and Chaperones*, vol. 14, no. 1, pp. 105–111, 2009.

[218] H. H. Kampinga and E. A. Craig, "The HSP70 chaperone machinery: J proteins as drivers of functional specificity," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 8, pp. 579–592, 2010.

[219] A. Fukushima, H. Kawahara, and C. Isurugi, "Changes in serum levels of heat shock protein 70 in preterm delivery and preeclampsia," *Journal of Obstetrics and Gynaecology Research*, vol. 31, no. 1, pp. 72–77, 2005.

[220] J. K. Park, T. G. Kang, M. Y. Kang et al., "Increased NFAT5 expression stimulates transcription of Hsp70 in preeclamptic placentas," *Placenta*, vol. 35, no. 2, pp. 109–116, 2014.

[221] F. Barut, A. Barut, B. Dogan Gun et al., "Expression of heat shock protein 70 and endothelial nitric oxide synthase in placental tissue of preeclamptic and intrauterine growth-restricted pregnancies," *Pathology - Research and Practice*, vol. 206, no. 9, pp. 651–656, 2010.

[222] Y. Liu, N. Li, L. You, X. Liu, H. Li, and X. Wang, "HSP70 is associated with endothelial activation in placental vascular diseases," *Molecular Medicine*, vol. 14, no. 9-10, pp. 561–566, 2008.

[223] H.-S. Cho, T. Shimazu, G. Toyokawa et al., "Enhanced HSP70 lysine methylation promotes proliferation of cancer cells through activation of Aurora kinase B," *Nature Communications*, vol. 3, article 1072, 2012.

[224] A. Sawano, T. Takahashi, S. Yamaguchi, M. Aonuma, and M. Shibuya, "Flt-1 but not KDR/Flk-1 tyrosine kinase is a receptor for placenta growth factor, which is related to vascular endothelial growth factor," *Cell Growth & Differentiation*, vol. 7, no. 2, pp. 213–221, 1996.

[225] N. Ferrara and R. S. Kerbel, "Angiogenesis as a therapeutic target," *Nature*, vol. 438, no. 7070, pp. 967–974, 2005.

[226] N. Rahimi, "Vascular endothelial growth factor receptors: Molecular mechanisms of activation and therapeutic potentials," *Experimental Eye Research*, vol. 83, no. 5, pp. 1005–1016, 2006.

[227] D. Hanahan and J. Folkman, "Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis," *Cell*, vol. 86, no. 3, pp. 353–364, 1996.

[228] W. Risau, "Mechanisms of angiogenesis," *Nature*, vol. 386, no. 6626, pp. 671–674, 1997.

[229] G.-H. Fong, J. Rossant, M. Gertsenstein, and M. L. Breitman, "Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium," *Nature*, vol. 376, no. 6535, pp. 66–70, 1995.

[230] S. Helske, P. Vuorela, O. Carpén, C. Hornig, H. Weich, and E. Halmesmäki, "Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies," *Molecular Human Reproduction*, vol. 7, no. 2, pp. 205–210, 2001.

[231] Y. Zhou, M. McMaster, K. Woo et al., "Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome," *The American Journal of Pathology*, vol. 160, no. 4, Article ID 62567, pp. 1405–1423, 2002.

[232] M. Kunizaki, R. Hamamoto, F. P. Silva et al., "The lysine 831 of vascular endothelial growth factor receptor 1 is a novel target of methylation by SMYD3," *Cancer Research*, vol. 67, no. 22, pp. 10759–10765, 2007.

[233] E. J. Hartsough, R. D. Meyer, V. Chitalia et al., "Lysine methylation promotes VEGFR-2 activation and angiogenesis," *Science Signaling*, vol. 6, no. 304, Article ID ra104, 2013.

[234] N. Rahimi and C. E. Costello, "Emerging roles of post-translational modifications in signal transduction and angiogenesis," *Proteomics*, vol. 15, no. 2-3, pp. 300–309, 2015.