

Review: Placental derived biomarkers of pregnancy disorders

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Abstract

Pregnancy is one of the greatest physiological challenges that a women can experience.

The physiological adaptations that accompany pregnancy may increase the risk of developing a number of disorders that can lead to both acute and chronic physiological

5 outcomes. In addition, fetal development may be impaired and, if the fetus survives, the child may be at an increased risk of disease throughout life. Pregnancy disorders are poorly

predicted by traditional risk factors and maternal history alone. The identification of biomarkers that can predict incidence and severity of disease would allow for improved and targeted prophylactic therapies to prevent adverse maternal and fetal outcomes. Many of

10 these pregnancy disorders, including preeclampsia, intrauterine growth restriction, gestational diabetes mellitus and preterm birth are known to be regulated at least in part by

poor trophoblast invasion and/or dysregulated placental function. Cellular stress within the placenta increases the release of a number of factors into the maternal circulation. While

15 many of these factors minimally impact maternal biology, others affect key physiological systems and contribute to disease. Importantly, these factors may be detected in

physiological fluids and have predicative capacity making them ideal candidates as biomarkers of pregnancy disorders. This review will discuss what is known about these

placental derived biomarkers of pregnancy disorders and highlight potential clinical opportunities for disease prediction and diagnosis.

Introduction

Maternal health complications that occur during pregnancy increase the risk of adverse health outcomes for both the mother and fetus. A number of factors are known to increase a women's risk of developing a pregnancy complication including advanced maternal age, poor nutrient intake, maternal obesity, poor cardiovascular or metabolic health, maternal parity and family/previous pregnancy history [1, 2]. Many women, however, develop a pregnancy complication spontaneously without any risk factors associated with the disease. Pregnancy disorders can severely impair maternal health and lead to persistent dysregulation of systemic physiology in the mother after pregnancy [3, 4]. Furthermore, the developmental trajectory of the fetus can be disrupted resulting in fetal loss. Alternatively, fetal development can be impaired and offspring may have an increased risk of developing a wide range of diseases in adulthood [5].

Most pregnancy disorders develop in late gestation and yet are thought to be caused by adverse regulation of trophoblast invasion and placental formation in early pregnancy [6]. Indeed, dysfunctional placental development is known to be involved in most pregnancy complications that often share similar mechanisms of origin [7]. It is important to note however, that while similarities exist and causative factors overlap, there are also major differences in the aetiologies of each pregnancy disorder. The placenta releases a number of factors into the maternal circulation and much research has investigated the potential role of many of these factors in disease diagnosis and early prediction. While most studies have investigated these factors in relation to a single pregnancy complication, many are likely to be associated with several common disorders of pregnancy. It is likely that markers common to multiple disorders will be useful as indicators of pregnancy adversity, while more specific markers might be associated with mechanistic changes unique to the pregnancy disorder in question. This review summarises the placental contribution to four common pregnancy disorders and highlights the possible use of a range of placental factors as biomarkers of disease.

Preeclampsia

One of the most common and life threatening disorders of pregnancy is preeclampsia [8].

Preeclampsia is a uniquely human condition of placental origin characterised by maternal endothelial cell dysfunction causing symptoms including hypertension and proteinuria.

- 5 Preeclampsia can have profound effects on maternal physiology and the only viable treatment strategy to prevent the onset of eclampsia (characterised by seizures which can eventually lead to coma and death) is removal of the placenta through induction of labour. The maternal symptoms of this condition are largely mediated by factors secreted by the placenta while fetal consequences of the condition are related to impaired placental function.
- 10 One such fetal consequence of preeclampsia is intrauterine growth restriction (IUGR). While IUGR does not occur in every pregnancy complicated by preeclampsia, women with preeclampsia have much higher rates of IUGR than women without preeclampsia[9].

IUGR

IUGR or fetal growth restriction occurs when the fetus fails to reach its expected growth

- 15 potential. These babies may be born small for gestational age (SGA) which is a term used to reflect fetal size below a prescribed cut off for any gestational age. While these two terms are often used interchangeably, they are not equivalent. Babies affected by IUGR are likely to show signs of placental disease and have worse perinatal outcomes compared to SGA babies [10]. Diagnosis of IUGR requires Doppler measurements be performed and
- 20 estimated fetal weights calculated [10]. IUGR is most commonly caused by placental insufficiency [11] and /or poor placental function with the placenta failing to make the adaptive changes required to maximise fetal growth [12, 13]. IUGR is strongly associated with perinatal mortality but is also known to increase the risk of a wide variety of diseases throughout the life course of the child [5]. Importantly, the risk of developing IUGR is also
- 25 associated with other pregnancy complications including gestational diabetes [14] and preterm birth [15].

Preterm birth

Approximately 10% of all pregnancies are known to conclude prematurely [16]. With preterm birth being one of the major causes of fetal death, it is important to identify women at risk of spontaneous onset preterm delivery before it occurs. Preterm birth is associated with higher rates of disability in children [17] and an increased risk of disease susceptibility throughout life [18]. While preterm birth is defined as birth less than 37 weeks of gestation, more severe outcomes are seen in infants that are severely premature. Preterm birth may be caused by multiple factors including inflammation and maternal stress, however, placental ischemia, and other forms of placental dysfunction commonly contribute [19]. Indeed, gestational length itself is known to be at least partially regulated by factors secreted by the placenta [20].

Gestational diabetes Mellitus

Gestational diabetes mellitus (GDM), which is defined as the onset of impaired glucose tolerance during pregnancy, occurs as a result of an impaired capacity of the maternal beta cells to adapt to the decreased insulin sensitivity that occurs during pregnancy. This inability to adapt is thought to be at least partially caused by insufficient production of placental hormones such as placental lactogens [21]. The increased glucose levels that occur during GDM can further impair placental development and fetal growth [22]. Interestingly, gestational diabetes can induce IUGR but more commonly leads to increased fetal growth and large for gestational age (LGA) fetuses and the placenta may play a role in determining which of these outcomes occur [23].

The placenta mediates both maternal and fetal consequences of adversity in pregnancy.

The placenta in fact plays a number of roles in mediating fetal and maternal outcomes in a number of pregnancy disorders [24]. Several key mechanistic pathways have been identified as contributing to disease progression with similar pathways affected in multiple pregnancy

disorders. Often these pathways are disrupted before the onset of maternal symptoms in conditions such as preeclampsia and gestational diabetes mellitus, or before the delivery of a growth restricted or prematurely born child. Importantly, therapeutic strategies that target these mechanistic pathways are likely to provide the best opportunity for disease prevention and treatment. It is important to note that changes to these pathways need to be detected as early as possible to allow for appropriate interventions to take place.

Placental biomarkers

Maternal symptoms of pregnancy complications are often caused by factors secreted by the placenta. These factors may either be “passive” markers of altered placental function or bioactive molecules secreted by the placenta that regulate maternal physiology. In healthy women, these bioactive peptides play an important role in inducing physiological adaptations required for a successful pregnancy. It is only when they are inappropriately regulated that disease symptoms present. These “passive” markers are often by-products of important placental processes that are detectable within the maternal system. As such they are often produced in response to the early developmental insults which precede maternal symptoms and so can be detected earlier in gestation and therefore may have some predictive value. In contrast, bioactive peptide concentrations within the maternal blood often change shortly before disease onset. These factors are likely to have strong diagnostic potential but have a somewhat more limited predictive capacity.

In every pregnancy disorder there are likely to be a range of placental factors that are detectable in the maternal circulation at different stages of disease progression.

Furthermore, although a single biomarker might be strongly associated with a disease, it is unlikely to be detectable in every woman who develops the condition. A number of reviews have listed potential biomarkers for predicting conditions such as preeclampsia [25, 26], however biomarkers are yet to be used diagnostically in routine clinical settings. Given the limited clinical assessment of biomarkers of complicated pregnancies, it is difficult to define the parameters (such as specificity, sensitivity or likelihood ratios) that are required to make

these biomarkers clinically useful. Indeed, a recent review highlighted that 401 different biomarkers of preeclampsia have been measured across 147 different studies of which specificity and sensitivity was only given for 36 studies [27]. A 2004 systematic review of markers of preeclampsia by the World Health Organisation (WHO) highlights that likelihood ratios are more clinically meaningful than specificity and sensitivity measurements in determining biomarker suitability [28]. This review recommends benchmarks for likelihood ratios to be greater than 10 for a positive test and less than 0.1 for a negative test with positive values less than 5 and negative values greater than 0.2 having only minimal predictive capacity. Using these parameters, the WHO reported that in 2004, no clinically useful biomarkers for preeclampsia prediction were available [28] and that future studies needed to change their study methods to be able to identify more clinically useful biomarkers. As of 2015, A WHO report demonstrated little progress with biomarkers (angiogenic markers) measured before 20 weeks of gestation having poor predictive potential for the later development of preeclampsia [29].

It is important to note that different factors within maternal fluids are likely to have different clinical benefits depending on gestational age in relation to the timing of disease onset. The current review is not an exhaustive list of biomarkers considered to date but a discussion around the origin of a range of these markers and their potential use as either predictive biomarkers or diagnostic tools for the above pregnancy complications. Many of the discussed markers have not been clinically assessed and further research is required to ascertain suitability for use in practice.

Placental stress markers

There are likely to be many factors secreted into the circulation long before disease presentation and these factors are often produced as a result of syncytiotrophoblast stress which is central to the aetiology of multiple pregnancy disorders [30, 31]. This multinucleated cell layer is vulnerable to a number of cellular stress pathways including oxidative stress, heat shock stress, endoplasmic reticulum stress, glucocorticoid exposure, hypoxia,

inflammation or nutritional stress. Induction of these stress pathways results in the release of stress-related markers into the maternal circulation. Both endogenous and environmental causes of many pregnancy complications converge on one or more placental stress pathways. The most commonly studied placental stress pathway known to be at the centre of many pregnancy disorders is oxidative stress. A recent review highlights the potential use of oxidative stress markers in the prediction and diagnosis of pregnancy disorders [32]. Many of the studies investigating cellular stress markers as biomarkers of pregnancy disorders unfortunately only measure these markers after disease diagnosis and so there is little evidence to support the theory that they would be preferred markers for early prediction of disease.

Markers such as protein carbonyls are stable by-products of tissue oxidation [33] which have been shown to be increased in the maternal serum of women with preeclampsia [34], GDM [35], IUGR [36] and preterm birth [37]. These studies investigated maternal protein carbonyl levels after disease diagnosis and so further research is needed to validate their use in disease prediction. Other markers such as oxidised DNA (8-oxo-7,8 dihydro-2 deoxyguanosine or 8 OH-dG) can be detected in maternal urine [38] and show promise as suitable biomarkers of pregnancy complications. 8 OH-dG levels in maternal urine at 16 weeks of gestation were found to be 26% higher on average in women who subsequently developed GDM with women having urinary levels higher than 8.01 ng/mg creatinine being 3.79 times more likely to develop GDM than women with levels less than 4.23 ng/ml creatinine [39]. 8-OHdG levels are also associated with IUGR in women at 28 weeks gestation [40]. Other studies have found strong links between oxidative stress products such advanced glycation end products (AGEs) [41] with multiple pregnancy complications, however clinical trials are needed to support their use as predictive markers.

In addition, placental markers of endoplasmic reticulum stress have been identified in placental tissue of GDM [42] and preeclamptic patients [43]. Glucose regulated protein 78 (GRP78) is a tissue marker of ER stress that is expressed by cytotrophoblasts [44] that can

be detected in maternal circulation. Laverrière et al demonstrated reduced levels of autoantibodies against GRP78 and a reduced ratio of C-terminal GRP78 to full length (FL) GRP78 in the serum of first trimester women who later developed preeclampsia. At term, while autoantibodies against GRP78 were still reduced, the ratio of C-terminal GRP78 to FL GRP78 was increased [44]. Another circulating marker of cellular stress known to be increased in pathological pregnancies is heat shock protein 70 (Hsp70). Hsp70 levels have been shown to be elevated in biological fluids in cases of preeclampsia and preterm birth (reviewed in [45])

Placental debris and extracellular vesicles

10 A number of important factors are deported from the placenta into the maternal circulation. The shedding of material from the placenta is part of normal pregnancy, however, the amount and nature of this material is known to change in pathological pregnancies. Excessive syncytiotrophoblast stress can result in the deportation of altered placental material which enters the maternal circulation where it may contribute to maternal disease symptoms [46]. In the last decade, there has been an explosion in research on extracellular vesicles of different types and sizes including nanovesicles, exosomes, microvesicles, and macrovesicles (reviewed in [47]). In addition, subcellular fragments including cell free DNA and RNA [48] and miRNA [49] have also been found in circulation. Further, large placental fragments may become lodged in the maternal lungs and may themselves be a source of smaller trophoblast vesicles [50].

Exosomes originate from the endosomal compartment via fusion of multivesicular bodies with the plasma membrane. In the context of pregnancy complications, placenta-derived exosome analysis can indicate placental metabolic state and function [51]. Extracellular vesicles are associated with a wide range of molecules including proteins, RNAs, and DNA, which may be useful diagnostically [52]. In exosomes, these molecules are protected by the lipid bilayer and represent a biomedical resource to identify biomarkers of placental function and pregnancy complications. Preeclampsia is also associated with increased DNA-positive

microvesicles [53], and altered lipid composition has been found in microvesicles in the maternal circulation during preeclampsia and in patients with a history of recurrent miscarriage [54, 55].

The concentration of placental vesicles in maternal circulation is greater in pregnancies affected by preeclampsia or GDM [56]. In preeclampsia, particle number does not correlate with disease severity [57] although higher levels have been reported in early onset-preeclampsia [58]. Acute placental injury may result in an intermittent increase in fragment shedding [59], leading to variable levels over time. Only a few studies have analysed vesicles in maternal plasma during early gestation in women who later develop pregnancy complications [60]. Recently, it has been established that the total number of exosomes present in maternal plasma was ~2-fold greater in women between 11 to 14 weeks who subsequently developed GDM (diagnosed between 22–28 weeks) compared to women who experienced normoglycemic pregnancies [56].

Subcellular fragments may also be of use for diagnosis. Pathological damage can lead to an adaptive increase in placental mitochondria, and alterations in mtDNA copy number in tissues (including blood) could provide a biomarker for disorders involving mitochondrial dysfunction. Indeed, increased levels of mtDNA have been detected in the blood of women with preeclampsia and IUGR [61, 62]. We suggest that changes in the profile of placenta-derived material may be of clinical utility in the diagnosis of placental dysfunction and the early identification of women at risk of developing complications of pregnancy.

Bioactive peptides

As described above, bioactive peptides produced by the placenta contribute to regulating maternal adaptations to pregnancy. The production of many of these biological peptides can be disrupted by placental stress and, therefore, can be used as biomarkers of pregnancy disorders. The majority of studies investigating the relationship between bioactive peptides and pregnancy disorders have focused on preeclampsia, however a number of these factors

are likely to be involved in a range of pregnancy complications. Soluble fms-like tyrosine kinase-1 (sFLT1), placental growth factor (PGF) and soluble endoglin (sENG) are all secreted by the placenta in response to syncytial stress and are three of the most widely studied biomarkers of preeclampsia [63]. While plasma sFLT1 levels are known to be increased in preeclamptic women, PGF levels are reduced. A recent systemic review of 28 populations demonstrated that the overall diagnostic accuracy of sFLT1/PGF for preeclampsia is relatively high, and highest for early onset preeclampsia. However, a high number of false negative (22%) and false positive (16%) outcomes were predicted, indicating only moderate accuracy that is insufficient for routine clinical application [64]. This highlights that while the sFLT1/PGF ratio might be increased in many women who develop preeclampsia, not all women who develop the disease will have increased sFLT1/PGF, and not every woman with a high sFLT1/PGF ratio will develop preeclampsia. While sFLT1 levels are known to be associated with preeclampsia, sFLT1 in maternal plasma most likely indicates placental disease. Indeed, sFLT1 levels have been reported to be elevated in maternal plasma of pregnancies complicated with IUGR [65] and late miscarriage [66]. A large international cohort study demonstrated increased plasma sENG concentrations in women who later develop early onset preeclampsia [67]. Studies have demonstrated that sENG concentrations are also associated with other pregnancy complications. Nergiz Avcioglu *et al.* demonstrated that maternal serum sENG concentrations were lower at delivery in women who had preterm premature membrane rupture compared to healthy control women at the same gestational age [68]. In contrast, a separate study demonstrated that sENG concentrations are higher in women 5-10 weeks prior to preterm delivery compared to controls [69].

Another group of peptides and related molecules that are increased in biological fluids of women with pregnancy disorders are components of the renin angiotensin aldosterone system (RAAS). This system is normally thought of as a mediator of fluid homeostasis outside of pregnancy. However, the placenta expresses all components of this system [70,

71] and key components of this system may regulate pregnancy and offspring outcomes following a maternal challenge [72]. Furthermore, a number renin angiotensin system peptides have been shown to be potential biomarkers of a range of pregnancy complications, particularly preeclampsia [26]. While angiotensin I and angiotensin II concentrations are known to increase over gestation, this does not occur in women with preeclampsia [73]. In addition agonistic auto-antibodies against the angiotensin II type 1 receptor are increased in preeclamptic women with IUGR [73] and studies have demonstrated that antibody mediated AT1 activation results in increased production of sFLT and sENG from trophoblast cells [74]. As such it is possible that AT1 autoantibodies might precede production of other commonly studied biomarkers making them a biomarker of interest requiring further investigation. A 2009 study demonstrated that serum concentrations of autoantibodies against AT1 correlated with preeclampsia severity and were present in 95% of the preeclamptic participants but were undetectable or at very low levels in normotensive women [75]. Increased circulating levels of (pro)renin or the (pro)renin receptor may also indicate a disorder of pregnancy with studies to date demonstrating elevated levels in women with preeclampsia [76]. Similarly, angiotensin II levels have been shown to be elevated in cord blood [77] of pregnancies affected by GDM. The smaller angiotensin fragment angiotensin 1-7 is reduced in pregnancies complicated by GDM [78] but increased in women with preeclampsia [79]. Components of this system are also implicated in preterm birth with Ang 1-7 again being reduced [80] compared to full term women. Like many other potential biomarkers discussed throughout this review, further studies need to measure components of the renin angiotensin system prior to disease onset and determine suitability for use as a clinical marker of disease.

As described above, placental lactogens play an important role in the beta cell expansion that occurs during pregnancy [21]. As such, the ratio of human chorionic gonadotropin to placental lactogen has been shown to be elevated in women with GDM [81]. This marker of GDM was part of a panel of markers trialled which had a positive detection rate of 87% in

women diagnosed with GDM [81]. This panel included a number of maternal glycoproteins such as fibronectin and pregnancy-specific glycoprotein 1 (PSG1). PSG1 is produced only during pregnancy by the syncytiotrophoblast with levels increasing over gestation. This study demonstrated that while PSG1 levels were not affected by GDM, glycosylated levels of
5 PSG1 was increased in women with GDM [81]. Leptin is another peptide known to be produced predominantly by the placenta during late pregnancy [82]. Cord blood leptin concentrations and placental leptin mRNA abundance have been shown to be reduced in IUGR babies and elevated in pregnancies complicated by either type 1 diabetes or GDM [83].

10 C-Type natriuretic peptide levels decline over gestation in healthy pregnancies but do not change in pregnancies complicated by a range of disorders [84]. Placental protein 13 (PP13) has also been shown to have some potential as a biomarker of pregnancy disorders. This placenta derived protein is a known modulator of immune function and is particularly associated with preeclampsia [85]. Corticotropin releasing hormone (CRH) is another
15 peptide of placental origin shown to be a possible biomarker of pregnancy complications. Given the role that CRH is thought to have in regulating parturition, it is predominantly studied in related to preterm birth [86] but has also be shown to be associated with other disorders. In a study in which plasma was collected at 33 weeks, CRH concentrations were significantly associated with an increased risk of preterm birth as well as fetal growth
20 restriction [87].

Detectable changes in many of the most commonly studied bioactive markers often occur only shortly before disease presentation suggesting that there is a need to investigate new and novel bioactive peptides for predictive purposes while symptomatic peptides may prove useful in monitoring disease progression. Studies investigating the peptide profile of
25 placental tissue from women with or without preeclampsia demonstrated that 8% of the peptide variance was attributable to preeclampsia and identified a number of novel peptides such as calcyclin [88]. Studies such as this provide an opportunity for the identification of

new biomarkers of pregnancy disorders. These newly identified factors must be detectable in maternal circulation and studies be performed in women earlier in gestation prior to the development of pregnancy disorders.

Summary

5 Complications of pregnancy, such as PE, GDM and IUGR, are diseases of placental origin. Therefore, molecules released from the placenta into maternal circulation provide an opportunity for early detection of these complications. To date, although numerous markers of placental origin have been strongly associated with pregnancy disorders, no biomarker has been successfully taken into clinical practice. Unfortunately, this is likely a consequence
10 of heterogeneity in disease aetiologies, timing of onset, erratic progression and severity of maternal symptoms. Furthermore, few studies have measured potential markers of disease prior to traditional disease diagnosis. Future studies must focus on the clinical translation of predictive markers of pregnancy disorders and measure how each of the markers discussed throughout this review change over the course of pregnancy in a range of women.

15 Identification of women at risk of developing pregnancy disorders during early gestation, before presentation of clinical symptoms, is likely to facilitate better clinical management, lessening the long term burden of pregnancy disorders on women's health and significantly reducing the programmed disease outcomes in children.

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