EDITORIAL

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Podocytes in Urine, a Novel Biomarker of Preeclampsia?*

Podocyty w moczu jako nowy biomarker stanu przedrzucawkowego?

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Abstract

Preeclampsia is a disorder occurring during pregnancy typically after 20 weeks of gestation. It affects both mother and unborn baby in at least 5–8% of all pregnancies. It is a rapidly progressive condition characterized by high blood pressure and the presence of protein in the urine. The symptoms, such as swelling, sudden weight gain, headaches and vision disturbances, are important signs of preeclampsia which can lead to maternal and infant illness and death. It is estimated that this disorder is responsible for 76,000 maternal and 500,000 infant deaths each year. The main hypothesis explaining the development of preeclampsia is the theory of placental hypoxia/ischemia. An imbalance between vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) seems to play a crucial role. Currently there is no way to predict, with certainty whether preeclampsia will develop during a given pregnancy. There is a need for a diagnostic tool which can help to identify and monitor women at risk. There is growing evidence that podocyturia – urinary excretion of viable podocytes may be a useful predictor of preeclampsia. This paper presents facts supporting such a hypothesis (**Adv Clin Exp Med 2013, 22, 2, 145–149**).

Key words: hypertension, podocytes, preeclampsia, pregnancy, proteinuria.

Streszczenie

Stan przedrzucawkowy to schorzenie pojawiające się podczas ciąży, zwykle po jej 20. tygodniu. Stan ten zagraża zarówno matce, jak i nienarodzonemu jeszcze dziecku, i dotyczy około 5–8% wszystkich ciąż. Stan przedrzucawkowy postępuje gwałtownie i charakteryzuje się współistnieniem nadciśnienia tętniczego i białkomoczu. Objawia się obrzękami, nagłym zwiększeniem masy ciała, bólami głowy i zaburzeniami widzenia, które mogą prowadzić do uszkodzenia płodu, a nawet śmierci matki i dziecka. Ogólnie szacuje się, że każdego roku na świecie z powodu stanu rzucawkowego umiera ok. 76.000 matek i 500.000 nienarodzonych dzieci. Główną hipotezą tłumaczącą powstanie stanu przedrzucawkowego jest teoria niedokrwienia/niedotlenienia łożyska. Kluczową rolę wydaje się

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grać zaburzenie równowagi między czynnikiem wzrostu śródbłonka naczyniowego (VEGF) a receptorem naczyniowego czynnika wzrostu śródbłonka (sFlt-1). Obecnie nie ma pewnej diagnostyki, która pomogłaby zapobiec rozwojowi stanu rzucawkowego. Znalezienie skutecznego narzędzia diagnostycznego, przydatnego w identyfikacji i monitorowaniu osób zagrożonych stanem przedrzucawkowym, wydaje się sprawą wielkiej wagi. Jednym z takich narzędzi z pewnością mogłoby być badanie wydalania podocytów z moczem. Poniższa praca przedstawia dowody na poparcie tej hipotezy (**Adv Clin Exp Med 2013, 22, 2, 145–149**).

Słowa kluczowe: białkomocz, ciąża, nadciśnienie tętnicze, podocyty, stan przedrzucawkowy.

Preeclampsia (PE) remains a leading cause of maternal and perinatal mortality and morbidity. It is defined as a coexistence of systolic blood pressure > 140 mm Hg or diastolic blood pressure ≥ 90 mmHg, as measured twice at least 6 h and less than 7 days apart, and proteinuria in 24-hour collection at least 0.3 g, repeated twice, developing after the 20th week of gestation among previously normotensive women, and the disappearance of these disorders until the 6th week after delivery [1].

PE is a multisystem disorder which affects 3.0–7.0% of healthy nulliparous and 1–3% multiparas [2]. About 10–15% of maternal deaths are associated with both preeclampsia and eclampsia.

The most common risk factors for PE are: first pregnancy (the most influential), hypertension during previous pregnancies, multi-fetal gestation, coexistence of diabetes, body mass index (BMI) ≥ 30, renal disease, maternal age > 40 (some authors underlay > 35), Afro-American ethnicity and family history [3].

It is a life threatening condition for both mother and child. The most serious maternal complications are eclampsia with tonic-clonic seizures, intarcerebral hemorrhage, HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) and neonatal outcomes as intrauterine growth restriction (IUGR), preterm labor and low birth weight [4]. Women affected by preeclampsia have an elevated risk of chronic hypertension, ischemic heart disease, stroke and venous thromboembolism [5]. It is also associated with the higher risk of hypertension, cardiovascular diseases and epilepsy in the offspring.

The pathophysiology of preeclampsia remains still unclear. The crucial role is played by placenta [6]. An important mechanism of preeclampsia is based on a theory of placental hypoxia/ischemia. This triggers the effector mechanisms responsible for maternal cardiovascular dysfunction, most notably oxidative stress, immunological dysfunction and an imbalance between proangiogenic and antiangiogenic factors. The most popular hypothesis underscores the role of immunological maladaptation between decidua and fetal trophoblast, which in consequence leads to systemic endothelial cells dysfunction and inflammatory response [7].

During normal pregnancy the villous cytotrophoblast invades the myometrium, and the spiral arteries lose their endothelium and most muscle fibers. These structural modifications are associated with functional alterations, turning spiral arteries into low-resistant, and thus less sensitive to vasoconstrictive substances. These ultrastructural changes are needed for adequate blood supply to the placenta and developing fetus. During preeclampsia, investigation of the placenta shows shallow trophoblast invasion and insufficient vessels' remodeling that leads to ischemia and overexpression of hypoxia-regulated genes. In laboratory studies restriction of uterine blood flow resulted in hypertension, proteinuria and endothelial dysfunction. Oxidative stress is a consequence of hypoxia and is a known factor affecting placenta. Peroxynitrite levels, a marker of oxidative stress, were found to be much higher among preeclamptic women than in normal gestation. Experimental studies explained relationship between placental ischemia and production of reactive oxygen species (ROS). The great importance in pathogenesis of preeclampsia has also been attributed to immunological dysfunction. There is strong evidence suggesting a possible autoimmunological character of preeclampsia. A higher risk of developing the disease by nulliparous women than by multiparous ones is in the frontline of evidence. Also repeated expression of paternal antigens in the fetus creates immune tolerance and reduces the incidence of preeclampsia. Another factor involved in preeclampsia is altered maternal inflammatory response. Based on experimental studies, the role of interleukin-6 (IL-6) and tumor necrosis factor – alpha (TNF-alpha) in above mentioned process has been proven. What should be also considered in the pathophysiology of preeclampsia is an imbalance between pro- and anti-angiogenic factors. Under hypoxia the placenta releases a number of vasoactive factors which are believed to play a crucial role in endothelium dysfunction, prominent in preeclampsia. The one which seems to have a particular importance is soluble fms-like kinase-1 (sFlt-1), a splice variant of vascular endothelial growth factor-1 (VEGF-1) receptor, the release of which has been shown in the trophoblast. SFlt-1 acts as antagonist of VEGF and placental growth factor (PlGF) altering their function by sequestering free protein in plasma. VEGF and PlGF produced by cytotrophoblast are main proangio-

genic factors responsible for proper development of the placenta. Levels of sFlt-1 both in plasma and in the placenta are elevated in preeclamptic women compared to normal pregnancy and their presence can indicate preeclampsia prior to disease manifestation.

A pathophysiological cascade triggered by placental hypoxia/ischemia increases levels of circulating angiotensin-1 receptor autoantibody (AT1-AA), TNF-alpha and soluble endoglin, subsequently detrimentally affecting several downstream targets. The imbalance between pro- and antiangiogenic factors such as sFlt-1, VEGF and PlGF and the increased production of ROS leads to the subsequent maternal endothelial dysfunction. Disorders in the endothelium are the main cause of clinical signs observed in the mother, i.e. impairment of hepatic endothelium (contributing to HELLP syndrome), impairment of cerebral endothelium inducing neurological disorders (seizures) and promotes microangiopatic hemolytic anemia. Endothelial dysfunction is also responsible for renal disorders, i.e. proteinuria or decrease of glomerular filtration rate (GFR). Elevated level of sFlt-1 and diminished levels of VEGF in plasma of hypertensive women are the main factors responsible for kidney injury. An experimental study conducted by Sugimoto et al. showed the development of significant proteinuria among mice which underwent an intravenous injection of anti-VEGF antibody [8]. The same authors performed also another experiment explaining the role of sFlt-1 in development of proteinuria. Experimental animals injected by single dose of sFlt-1 also developed significant proteinuria. In addition, supplementation of VEGF in concentration which compensates injected sFlt-1, inhibited proteinuria. Analysis performed with the use of electron microscopy on tissue samples processed from kidneys of animals which developed proteinuria, revealed glomerular hypertrophy, endothelial cell detachment from glomerular base membrane (GBM) or disruption of slit diaphragm. the above mentioned changes were similar both for animals treated with anti-VGEF antibody and sFlt-1.

The link between renal involvement in preeclampsia and anti-angiogenic factors responsible for PE development is localized in podocytes, which are the major glomerular source of VEGF [9]. In 2003, Foster et al. showed autocrine functions of VEGF released by podocytes and underlined its importance in glomerular barrier integrity [10]. Enhanced VEGF expression has been found in kidney tissue specimens possessed from women suffering from PE [11].

Screening women at high risk and preventing recurrences are key issues in the management of preeclampsia. Regardless of unknown origin, lack of knowledge on prophylaxis and therapeutic agents used against preeclampsia, it is extremely important to search for noninvasive, easily determined biomarkers, enabling the prediction or detection of this life-threatening disorder.

Based on pathophysiological hypothesis of preeclampsia, the main interest has been focused on placental factors and on the maternal proteins. Among many potentially useful biochemical markers, the outstanding importance has been attached to: soluble fms-like tyrosine kinase 1 (sFlt-1), soluble Endoglin (sEng), P-Selectin, cell-free fetal DNA, disintegrin and metalloprotease 12 (ADAM 12), pentraxin 3 (PTX3) and adrenomedullin [12]. Many authors underline also the role of urinary podocytes as a potential marker of preeclampsia.

Petermann and colleagues isolated podocytes from urine collected from rats with experimental model of membranous nephropathy (passive Heyman nephritis) which were viable and possessed the ability to grow in culture [13]. Yu et al. showed podocyturia to be a more specific marker for disease activity, especially glomerulonephritis than proteinuria and that podocyturia is more characteristic for active phase of injury, in contrast to proteinuria, which is present in both active and chronic phase [14]. Based on these findings several papers exploring the role of podocyturia in both primary and secondary glomerular diseases have been published. The role of urinary podocytes has been established in IgA nephropathy, focal segmental glomerulosclerosis, and membranous nephropathy but also in lupus nephritis, hemolytic uremic syndrome and diabetic nephropathy [15–19]. Garovic et al. isolated podocytes from urine in preeclamptic women. Authors showed strong correlation between podocyturia and proteinuria. They also proved that the number of podocytes excreted in urine is a sensitive marker for renal damage and protein excretion among patients with preeclampsia. The other finding was that urinary podocytes were absent in healthy pregnant women or even those with hypertension but without any other signs of preeclampsia [20]. Aita et al. found a strong correlation between the number of podocytes lost in urine and blood pressure but not with proteinuria [21]. In 2011, Zhao et al. found a diminished expression of podocyte slit diaphragm proteins like nephrin, podocin and polarity proteins in preeclampsia [22].

Although urinary podocytes are a very promising marker not only for preeclampsia but also for other, both primary and secondary glomerulopathies, there is an issue which makes the whole story more complicated.

The question of great importance is how to accurate identify isolated cells. As reviewed by

Skoberne et al., there is no good single marker for podocytes [23]. Among many available antigens, podocalyxin (PDX) seems to be the most robust. According to Vogelmann et al., cells positive for PDX were positive for other podocyte specific markers only in 30–40% [24]. On the other hand, all the cells staining positively for other podocyte markers were also positive for PDX but, as showed by Achenbach and colleagues, cells retrieved from urine, which are PDX positive are not only podocytes [25]. Parietal Epithelial Cells (PECs) share with podocytes embryogenic origin and express of antigens characteristic for mature podocytes, i.e. podocalyxin, GLEEP-1, synaptopodin, podocin and nephrin but don't express cytokeratin. Besides podocytes, PDX is also present on platelets or megakaryocytes. In the latest paper by Mueller-Deile, the authors assessed only PDX-positive cells without distinguishing them between podocytes and parietal epithelial cells [26]. This study shows a strong positive correlation between the number of PDX-positive cells excreted in urine among patients with focal segmental glomerulosclerosis (FSGS) and the negative change in serum creatinine concentration, suggesting their regenerative potential. This may be in contrast to previous studies, where the high number of PDX positive cells was associated with disease activity and poor prognosis.

Other podocyte specific proteins, like nephrin and podocin, contribute to the integrity of the filtration barrier and are highly downregulated in nephrotic syndrome [27]. Another possible marker for podocyte identification is synaptopodin – a protein of cytoskeleton. Like nephrin and podocin, its expression is also strongly diminished in proteinuric conditions [28]. Wilms tumor protein (WT1), a zinc finger protein which regulates the transcription of PDX, is downregulated in collapsing form of FSGS [29]. Interestingly, in cited above paper by Vogelmann et al. cells positive for all other podocyte markers (podocalyxin, podocin, synaptopodin and GLEEP1 were WT-negative.

As far as delivery is the only curative treatment for pre-eclampsia, there is a need to find a useful diagnostic tool, to identify and monitor patients at risk and thus provide the best prenatal care. The search for non-invasive test that could predict the development or assist in detection of this life-threatening disorder is still of utmost importance. Among many others – urinary podocytes are very promising.

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