Angiotensin Converting Enzyme 90 kDa isoform: Biomarker for diagnosis of preeclampsia? ☆

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ABSTRACT

Preeclampsia (PE), one of the leading gestational hypertensive diseases, is characterized by increased blood pressure ($\geqslant$140/90 mm Hg) and pathological proteinuria after 20 weeks gestation. It is a complex, multifactorial syndrome with an unestablished etiology and cure. The search continues for a biomarker that could assist in the early prediction or diagnosis of PE, reducing the rate of maternal and fetal mortality. Based on the findings of Casarini et al. that suggest the 90 kDa isoform of the Angiotensin Converting Enzyme (ACE) as a possible marker of hypertension, we hypothesized that this isoform may be present in pregnant women with PE, since they present a transient and spontaneous model of systemic arterial hypertension in pregnancy.

We believe, therefore, that pregnant women with pure PE (PPE) express the ACE 90 kDa isoform in urine, as well as having elevated isoform enzymatic activity, during pregnancy only. Postpartum, with the normalization of blood pressure, the protein isoform would no longer be expressed. Pregnant women with superimposed preeclampsia (SPE) would present the ACE 90 kDa isoform both during and after the gestation period, and its enzymatic activity would remain high as they are chronically hypertensive. It is expected that normotensive pregnant women do not present this isoform in their urine as elevated blood pressure levels do not occur. Both normotensive and PPE affected pregnant women with a family history of hypertension, will possibly express the ACE 90 kDa isoform before pregnancy and may become hypertensive, only after some years, through the influence of environmental factors and/or other diseases.

If our hypothesis is confirmed, it will allow differentiation of PPE and SPE sooner than 12 weeks postpartum, which is currently the estimated period for confirmation of the specific diagnosis. Furthermore, it could be an early biomarker for predicting the disease, enabling the physician to choose the best clinical management. In addition, it would minimize the use of other methods as the biological sample for obtaining the marker is urine, a practical and effective test with good reproducibility. Finally, test results would enable a greater understanding of the mechanisms involved in gestational hypertension.

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Introduction

Preeclampsia (PE), is considered one of the leading causes of maternal and fetal morbidity and mortality in the world, affecting around 2–8% of pregnancies [1]. It is characterized by the presentation of blood pressure levels $\geqslant$ 140/90 mm Hg and proteinuria after 20 weeks gestation [2,3]. PE has been known as "the disease of theories", mentioned by Lindheimer et al. [4]. Several mechanisms are involved in its pathophysiology, including the involvement of immunologic [5], inflammatory [5,6], renal ischemia [7], and vascular factors, among others [2,8–10]. Although its cure remains unknown, it is recognized that the placenta is directly involved in the syndrome outcome, since its removal results in the disappearance of clinical manifestations [11,12].

International consensus on the best criteria for defining preeclampsia has not yet been reached [3,13–19]. As per guidelines, PE is classified as: preeclampsia – expressed through elevated blood pressure and proteinuria after 20 weeks gestation, with

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values returning to normal up to 12 weeks postpartum, and in this text we will refer to as pure PE (PPE); superimposed preeclampsia (SPE) – pregnant women with preexisting chronic hypertension, presenting with or without proteinuria, that exhibit a sudden increase in proteinuria after 20 weeks gestation and continue hypertensive postpartum [3]. The term PE syndrome (PES) has been proposed for circumstances when it is not possible to differentiate between PPE and SPE [20].

Efforts have been made to obtain a sensitive and specific biomarker for early diagnosis of PES, enabling accurate prediction in the first trimester of pregnancy. This would assist in prevention or treatment of the disease, and reduce the rate of maternal and fetal morbidity and mortality. Several molecules have been proposed for assisting in PE diagnosis, however, it is a complex and multifactorial syndrome, and as such, no ideal biomarker has yet been obtained [2,16,21,22].

In recent years, Levine, Karumanchi, Maynard and coworkers have demonstrated the involvement of proangiogenic factors (PIGF-placental growth factor; VEGF-vascular endothelial growth factor) and antiangiogenic factors (sFlt-1-soluble fms-like tyrosine kinase 1 and sEng-soluble endoglin) in the placentation process, and are being considered promising for the early risk identification of PE before 34 gestational weeks [23,24]. Currently, the PIGF/sFlt1 ratio has been proposed as one of the best methods for predicting the disease [22].

The renin-angiotensin system (RAS), responsible for the regulation of blood pressure and electrolyte homeostasis, has been actively investigated in PES [25,26]. Studies on the genetic polymorphism of the angiotensin-converting enzyme (ACE), one of the components of the RAS, demonstrate that the D allele is related to elevated plasma ACE levels, and is strongly associated with cardiovascular diseases [27]. However, the findings concerning ACE genetic polymorphism in PE are contradictory [28–32].

Casarini et al. described the presence of ACE isoforms expressed by molecular weights of 190 and 65 kDa in the urine of normotensive individuals, differing from those with molecular weights of 90 and 65 kDa found in hypertensive patients, which are enzymes known as N-domain ACE isoforms [33]. This finding has been proposed as a urinary marker of hypertension, evaluating the presence of the 90 kDa isoform [34]. Therefore, the involvement of this isoform in the pathophysiology of gestational hypertension is questioned herein.

Hypothesis

It is hypothesized that the 90 kDa protein ACE isoform is present in the urine of pregnant women with SPE, before and after pregnancy. Its enzymatic activity is raised during the gestation period, and remains postpartum as the patient continues to be hypertensive.

In pregnant women with PPE its expression occurs only during the gestational period, except in women with a family history of hypertension. It is assumed that the enzymatic activity of the isoform is increased during pregnancy and normalizes postpartum.

Normotensive pregnant women with no family history of hypertension do not express the 90 kDa ACE isoform during and after the gestational period, and therefore, would have no enzymatic activity as no increase in blood pressure levels takes place.

Evolution of the hypothesis

90 kDa isoform – a biomarker of hypertension

The presence of the 65 kDa and 190 kDa (somatic ACE) isoforms have been described in the urine of normotensive rats and humans, while the 90 (N-domain ACE) and 65 kDa isoforms have been encountered in the urine of hypertensive rats and humans, and in normotensives with a family history of hypertension. According to these studies, the 90 kDa ACE isoform may contribute to the early diagnosis of hypertension, assisting in the prevention of cardiovascular diseases [33–38].

PES, characterized as a spontaneous and transient human model of hypertension in pregnancy, is a multisystem disorder that has great impact on maternal and fetal health [39]. In line with the scientific literature, and based on the assumption that the 90 kDa ACE isoform is associated with essential hypertension [33–36], our hypothesis is formed of the following possibilities, as illustrated in Fig. 1:

○ Normotensive pregnant women: we believe they do not present the 90 kDa ACE isoform during and after pregnancy as they maintain normal blood pressure levels. However, women with a family history of systemic arterial hypertension may express the 90 kDa ACE isoform in their urine and may become hypertensive in the future, influenced by various environmental factors or diseases. It is, therefore, supposed that the enzymatic activity of the 90 kDa ACE isoform is null during the gestational period, since it is not present in normotensive women with no family history of hypertension.
○ Pregnant women with PPE: we believe they present the 90 kDa ACE isoform during pregnancy due to increased blood pressure, with the isoform no longer being expressed in the urine postpartum as blood pressure levels return to normal. In cases where women have a family history of hypertension, they may have protein expression before pregnancy, evolving to PE and then normalizing their clinical condition after delivery. These women can become hypertensive in the future due to external factors or other diseases. Thus, the enzymatic activity

	NORMAL PREGNANCY		PURE PREECLAMPSIA		SUPERIMPOSED PREECLAMPSIA	
	Gestation	Postpartum	Gestation	Postpartum	Gestation	Postpartum
90 kDa	X	X	90 kDa	X	90 kDa	90 kDa
Enzyme Activity	∅	∅	↑	∅	↑	↑

∅ : null; ↑ : elevated; X :absent.

Fig. 1. Enzyme activity and Angiotensin Converting Enzyme 90 kDa isoform in the urine of normal pregnant and women with preeclampsia.

of the 90 kDa ACE isoform in patients with no history of systemic arterial hypertension should be increased during the gestational period, disappearing in the puerperium.
○ Pregnant women with SPE: these women must already express the 90 kDa ACE isoform in their urine as they are chronically hypertensive. Therefore, this group would present expression of this isoform before and after pregnancy due to essential hypertension. The same should occur with enzyme activity, with it remaining increased during and after the gestational period.

A comparative study should be conducted between pregnant women, normotensive and with PES, and being more than 20 weeks pregnant, in order to evaluate the association between the protein polymorphism of ACE and the PPE and SPE classes. The same is applicable for the activity of this enzyme in the urine, as proposed in Fig. 1. This experimental design, associated with the results connected to our hypothesis, would differentiate between PPE and SPE in the immediate postpartum period, something that is normally done 12 weeks after delivery when the hypertension present in SPE does not disappear. In this way, the patient with SPE would have early confirmation of the diagnosis of essential hypertension, emphasizing the importance of maintaining permanent follow-up. In addition, the results would make it possible to detect if the mechanisms involved in essential hypertension are the same as in gestational hypertension.

Pregnant women are, in general, young ladies who are at a reproductive age and do not suffer from chronic diseases, such as hypertension. After delivery, they are inclined to not adhere properly to clinical monitoring. Therefore, the diagnosis of PPE by the biomarker proposed in Fig. 1, would suggest a differentiated clinical management for patients with SPE, showing the importance of controlling blood pressure levels through occasional medical follow-up, since they would have been warned of the relevance of this monitoring. It is reported that women with PE show greater risk of manifesting cardiovascular and renal disease in the future [40–42].

The presence of the 90 kDa ACE isoform and the increase in the ACE enzymatic activity in the postpartum period of women with PPE, suggests a family history of hypertension. This would indicate the same medical management as that given to women who do not present 90 kDa ACE isoform after delivery.

Urinary detection of the 90 kDa ACE isoform

In order to confirm our hypothesis and based on the experimental methods used by Casarini et al. [34,38], two urine collections should be made from pregnant women with PES, to analyze the presence and enzymatic activity of the 90 kDa ACE isoform: the first sample after 20 weeks gestation, and the second within 12 weeks after delivery.

It would be interesting to analyze studies comparing genetic polymorphism with protein polymorphism and the enzymatic activity of ACE isoforms in urine, to observe whether there is correlation between these results. Once the gene is activated, it produces a protein by as yet unknown mechanisms, which creates different isoforms in normotensive and hypertensive individuals, giving contrasting measurements of enzymatic activity. These results could help in understanding this gene-protein-enzyme activity route. Meanwhile, the findings related to the genetic polymorphism of ACE in PE results in different data. Perhaps this contradiction is based on the analysis of distinct populations, with them having differing ethnic and genetic characteristics [28–32].

Enzyme activity of the 90 kDa isoform

The enzymatic activity of ACE isoforms is an additional analysis to confirm the presence of these proteins in urine, through their measurement using two substrates: Hippuryl-His-Leu (HHL) [39] to quantify the somatic ACE with 190 kDa and Z-Phe-His-Leu (ZPhe-HL) [43,44] to quantify the 90 and 65 kDa N-domains isoforms, as outlined in a technique by Casarini et al. [34,38]. Determining the ZPhe-HL/HHL activity ratio indicates the highest quantity/activity of the N-domain isoform (90/65 kDa).

Consequences of the hypothesis and discussion

Many studies have been performed in an attempt to find a biomarker that may assist in the diagnosis of PE; enabling the physician to choose the best clinical practice for the welfare of the patient, and preventing serious progression of the pathological condition. However, to date, many of the proposed molecules have proven not accurate or sensitive enough in predicting the disease. The use of urine as the biological sample for the detection of the 90 kDa ACE isoform would be a low-cost, highly reproducible and effective clinical examination. All patients could have access to this clinical analysis. An ideal biomarker should be one that is not obtained through invasive methods and should be practical, effective and sensitive. Thus, if the results confirm our hypothesis, the 90 kDa ACE isoform may be an excellent biomarker for the specific diagnosis of PE.

Conflict of interest statement

The authors declare no conflict of interest in relation to financial and personal relationships with other people or organizations that could inappropriately influence this work.

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