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Tissue kallikrein and kininogen levels in fetoplacental tissues from normotensive pregnant women and women with pregnancy-induced hypertension

Mahaneem Mohamed [a,*], Ernest T. Larmie [a], Harbindar J. Singh [a], Mohd. S. Othman [b]

[a] *Department of Physiology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia 16150, Kubang Kerian, Kelantan, Malaysia*
[b] *Department of Obstetric and Gynecology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia 16150, Kubang Kerian, Kelantan, Malaysia*

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Abstract

Objectives: An imbalance of vasoconstrictor and vasodilator substances in the placenta has been postulated in the pathogenesis of pregnancy-induced hypertension (PIH). There is however little information available on the kallikrein-kinin system (KKS) in women with PIH. The aim of this study therefore was to determine tissue kallikrein and kininogen levels and their distribution patterns in fetoplacental tissues from both normotensive pregnant (NTP) women and women with PIH.
Study design: The study group consisted of 24 women, 12 of whom had normal pregnancies, while 12 had PIH. Portions of amnion, chorion laeve, placental plate chorion, fetal placenta and maternal placenta were dissected from each freshly delivered placenta. Tissue kallikrein (total, active and inactive) and kininogen levels were estimated using a synthetic chromogenic substrate, S-2266 and an enzyme immunoassay, respectively. The data were analysed using Mann–Whitney *U* and Kruskal–Wallis tests.
Results: No significant differences were found for total, active and inactive tissue kallikrein levels in all fetoplacental tissues between both groups. However, kininogen levels were found to be significantly lower in chorion laeve, placental plate chorion, fetal placenta and maternal placenta from women with PIH when compared to those in similar tissues from NTP women.
Conclusion: These findings suggest the presence of an abnormality in the kallikrein-kinin system in the placentas of women with PIH, which requires further study.

Keywords: Placenta; Tissue kallikrein; Kininogen; Pregnancy-induced hypertension

1. Introduction

Pregnancy-induced hypertension (PIH) is the most common medical disorder of pregnancy and remains a major cause of maternal and perinatal morbidity and mortality worldwide. Although its pathogenesis remains an enigma, it is nevertheless widely accepted that the abnormality in this disorder lies in the placenta, as the disease usually abates soon after the delivery of the placenta. The precise placental abnormality remains unknown but it is postulated that there is a reduction in placental perfusion in PIH that results in a release of a stress factor(s) into the maternal circulation causing a generalized maternal endothelial dysfunction resulting in multiple pathophysiological changes and subsequent clinical manifestations of PIH [1,2].

Since the placenta lacks nervous innervation, placental perfusion might be regulated mainly by the action of vasoactive agents released either locally or from systemic circulation [3,4]. Consequently, an imbalance of vasoconstrictor substances over vasodilator substances within the placenta might be a contributing factor in the placental hypoperfusion as seen in PIH. This assertion is supported by

* Corresponding author. Tel.: +60 9 7664826; fax: +60 9 7653370.
E-mail address: mahaneem@kck.usm.my (M. Mohamed).

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reports of higher levels of vasoconstrictor substances such as thromboxane [5] and endothelin-1 [6] coupled with lower levels of a vasodilator such as prostacyclin [5], and nitric oxide synthase, an enzyme required for the production of nitric oxide, another vasodilator [7] in the placentas of women with PIH compared to those in normal pregnancy.

The presence of kininogen [8], tissue kallikrein [9], their respective mRNAs [9,10] and kinin B_2 receptors [11] has been reported in normal human fetoplacental tissues. These findings suggest a local production of kinin, a potent vasodilator that has a very short half-life, within placental circulation that might play a role in regulating the placental blood flow. Although the presence of these components of kallikrein-kinin system (KKS) has been reported in the placenta in normal pregnancy yet to our knowledge, no study has been done to determine whether there is any alteration in these components in placentas from women with PIH. We therefore determined the levels and the distribution patterns of tissue kallikrein (total, active and inactive) and kininogen in fetoplacental tissues from normotensive pregnant (NTP) women and women with PIH.

2. Materials and methods

Fresh placentas were obtained from 12 NTP women and 12 women with PIH following normal vaginal deliveries. The subjects were matched for age and gestation and the placentas were collected after obtaining informed consent from all the subjects. The inclusion criteria for women with PIH were (i) a diastolic blood pressure of $\geq$110 mmHg on one occasion or $\geq$90 mmHg on two occasions, taken 4 h or more apart where the increase in blood pressure should have developed after 20 weeks of gestation with the presence or absence of proteinuria and (ii) the high blood pressure and abnormal urine must return to normal values within 6 weeks after delivery [12,13]. Women with diabetes mellitus, renal disease or multiple pregnancies were not included in the study. Normal placentas were collected from women with no history of any diseases and with normal blood pressure and without proteinuria throughout pregnancy.

Baseline data consisting of maternal age, gestational age, systolic blood pressure and diastolic blood pressure before delivery and neonatal birth weight were recorded. Portions of amnion, chorion laeve, placental plate chorion, fetal placenta (fetal surface of placenta) and maternal placenta (surface of placenta attached to the uterus) were dissected from each placenta [6]. Each tissue was immediately washed thoroughly with Krebs–Henseleit (KH) buffer, pH 7.4, dried between filter papers, weighed and frozen in buffer solution at −80 °C for analysis at a later date [14].

Tissue samples for the estimation of tissue kallikrein and kininogen levels were prepared as described by Sharma et al. [14]. Briefly, each tissue was separately homogenized in KH buffer (200 mg tissue/ml of buffer). After centrifugation, the supernatant was dialysed overnight at 4 °C against Tris–HCl buffer, pH 7.4. The dialysed supernatant was used as a sample for active and total tissue kallikrein, and kininogen estimation. Active tissue kallikrein level was estimated by the amidolytic activity of tissue kallikrein on a specific synthetic chromogenic substrate, S-2266 (H-D-Val-Leu-Arg-pNa from Chromogenix SBU, Italy). Briefly, 50 μl of dialysed sample was added to 2250 μl of preincubated 0.05 M Tris–HCl buffer at 37 °C, followed by the addition of 200 μl of 2 mM S-2266 after 2 min. The increase in absorbance was measured spectrophotometrically (UV/VIS Spectrophotometer from Jasco, Japan) at a wavelength of 405 nm after 4 min. The optical density obtained for the various samples were used to determine the levels of tissue kallikrein from a concurrently derived standard curve.

Total tissue kallikrein was determined after trypsin activation of the inactive tissue kallikrein in the supernatant. Briefly, 20 μg of trypsin (Sigma, USA) was added to 500 μl of the dialysed sample. An aliquot of 50 μl of trypsinized sample was then treated as described above for the determination of active tissue kallikrein. The difference between total and active tissue kallikrein was calculated as inactive tissue kallikrein. The results are expressed as kallikrein unit in 1 mg of fetoplacental tissue (kU/mg).

Kininogen level was determined after trypsin activation of both low and high molecular weight kininogens in the supernatant to generate kinin. Briefly, 1 ml of the diluted dialysed supernatant (1:5 dilutions) in a test tube was first placed in a water bath at 100 °C for 10 min. After cooling, the sample was incubated for 60 min with 20 μg of trypsin (Sigma) in the presence of 3 mM 1–10 phenanthroline (Sigma) at 37 °C, to generate kinin. The reaction was stopped by the addition of 100 μg soya bean trypsin inhibitor (Sigma) and incubated again for 20 min. Following this, the test tube containing the mixture was placed in a water bath at 100 °C for 2 min. Then, 0.5 ml of the sample was added to 0.1 ml of 20% trichloroacetic acid, thoroughly mixed and centrifuged at 3000 rpm for 10 min at 4 °C. The released kinin in the supernatant was then measured by enzyme immunoassay (competitive binding technique) using Markit-M Bradykinin Kit (Dainippon Pharmaceutical, Japan). Briefly, 100 μl of bradykinin antibody was added to each microplate assay well and incubated for 1 h at room temperature. The wells were then washed with the wash buffer solution and 100 μl of the prepared supernatants from each tissue were added to the wells and left to incubate for 1 h at room temperature. After this, 50 μl of bradykinin enzyme conjugate was added to each well and allowed to stand overnight at 4 °C. The contents of the wells were drained and the wells were washed with the wash buffer. After draining, 100 μl of the substrate solution was added to the well and allowed to stand at room temperature for 30 min. Stop reagent (100 μl) was added, mixed and incubated for another 30 min at room temperature after which the absorbance was read at 490 nm using a microplate reader (Dynatech Lab, UK). Kininogen levels are expressed as pg bradykinin equivalent/mg tissue [14]. The intra-assay