International Journal of Gynecology and Obstetrics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Gynecology and Obstetrics

journal homepage: www.elsevier.com/locate/ijgo

CLINICAL ARTICLE

Regional differences in the placental levels of oxidative stress markers in pre-eclampsia

Akriti S. Sahay [a], Deepali P. Sundrani [a], Girija N. Wagh [b], Savita S. Mehendale [b], Sadhana R. Joshi [a,*]

[a] Department of Nutritional Medicine, Interactive Research School for Health Affairs, Bharati Vidyapeeth University, Pune, India
[b] Department of Obstetrics and Gynecology, Bharati Medical College and Hospital, Bharati Vidyapeeth University, Pune, India

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form 16 December 2014
Accepted 25 February 2015

Keywords:
Catalase
Glutathione peroxidase
Malondialdehyde
Oxidative stress
Placenta
Pre-eclampsia

ABSTRACT

Objective: To examine placental malondialdehyde (MDA), catalase, and glutathione peroxidase (GPx) levels in four placental regions among women with and without pre-eclampsia. *Methods:* A cross-sectional study was conducted among women aged 18–35 years with a singleton pregnancy in Pune, India, between May 3, 2013, and June 16, 2014. Three groups were enrolled: normotensive; pre-eclampsia, delivered at term; and pre-eclampsia, delivered preterm. Samples were collected from the central and peripheral placental regions (maternal and fetal sides) immediately after delivery. *Results:* A total of 60 women were enrolled (35 normotensive; 11 with pre-eclampsia delivered at term; 14 with pre-eclampsia, delivered preterm). MDA levels were higher in all regions of the placenta among the pre-eclampsia versus normotensive groups ($P < 0.01$). MDA levels were higher in the central maternal region than in the central fetal region in the preterm pre-eclampsia group ($P = 0.023$). The MDA levels in the central maternal region were also higher in the preterm than in the term pre-eclampsia group ($P = 0.014$). Catalase activity was lower in the peripheral maternal ($P = 0.036$) and fetal ($P = 0.050$) regions in the preterm pre-eclampsia group versus the normotensive group. The activity of GPx was higher in the peripheral maternal region than in the central fetal region in the normotensive group ($P = 0.033$). *Conclusion:* Pre-eclampsia might be characterized by differential placental oxidative stress and antioxidant enzyme activity.

1. Introduction

Pre-eclampsia is a multisystem disorder that represents a major cause of maternal and perinatal morbidity and mortality worldwide [1]. The condition is characterized by new-onset hypertension and proteinuria after 20 weeks of pregnancy [2]. Several studies have demonstrated a causal relationship between oxidative stress and the pathophysiology of pre-eclampsia [3,4].

Oxidative stress arises from a disparity between the generation of potentially damaging levels of reactive oxygen species and the antioxidants that prevent their harmful effects. This imbalance can cause modifications and damage to various molecules, including lipids, proteins, and DNA [5]. Malondialdehyde (MDA) is a naturally occurring by-product of lipid peroxidation; consequently, this organic compound is an important indicator of oxidative stress, and high placental levels of MDA have been reported among women with pre-eclampsia [6,7].

Although the underlying cause of pre-eclampsia is unknown, abnormal placentation has been suggested to contribute to the pathophysiology [8]. Catalase and glutathione peroxidase (GPx) are antioxidant enzymes that confer cellular protection from oxidative stress. Several small studies have examined the activity of these enzymes in placental samples from pregnant women with or without pre-eclampsia [4,9,10]. The results obtained were inconsistent: some studies reported reduced placental catalase activity among women with pre-eclampsia [9,10], whereas others found increased activity [4]. One investigation reported reduced placental GPx expression and activity in association with pre-eclampsia [9]. However, these studies did not specify the region of the placenta that was examined. The placenta is a complex structure of heterogeneous function [11], so the inconsistencies reported in these previous studies might reflect sampling errors. Furthermore, different regions of the placenta can vary in tissue architecture, oxygen availability, and indices of oxidative stress [12–14].

Gene expression can be regulated in response to changes in oxygenation through the activity of transcription factors that specifically respond to the oxidation and reduction status of a cell. In addition, the stability of messenger RNA transcripts can be altered by a low oxygen environment [15]. This situation might lead to regional differences in the expression of antioxidant enzymes, such as catalase and GPx, as well as other genes involved in the development of the placenta, which eventually affect fetal development.

* Corresponding author at: Department of Nutritional Medicine, Interactive Research School for Health Affairs, Bharati Vidyapeeth University, Katraj-Dhankawadi, Pune 411043, India. Tel.: +91 020 24366929; fax: +91 020 24366931.
E-mail address: srjoshi62@gmail.com (S.R. Joshi).

http://dx.doi.org/10.1016/j.ijgo.2015.03.001
0020-7292/

One previous study [11] has shown changes in protein expression and activity of GPx in different regions of the placenta, but it was limited by a small sample size (n = 12). The activity of placental antioxidants might further vary between women with pre-eclampsia who deliver at term and those who deliver preterm as the pathology leading to preterm pre-eclampsia is more severe than is that of term pre-eclampsia [16].

Previous studies at the Interactive Research School for Health Affairs, Bharati Vidyapeeth University, Pune, India, also suggest the existence of different subsets of pre-eclampsia (term pre-eclampsia and preterm pre-eclampsia) [17,18]. Therefore, the objective of the present study was to examine MDA, GPx, and catalase in four distinct regions of the placenta among normotensive women and women with pre-eclampsia (term or preterm).

2. Materials and methods

A cross-sectional study was conducted among pregnant women who attended the Department of Obstetrics and Gynecology at Bharati Medical College and Hospital, Pune, India, between May 3, 2013, and June 16, 2014. Individuals aged 18–35 years with a singleton pregnancy complicated by pre-eclampsia were enrolled. Exclusion criteria were any other pregnancy complication (i.e. chronic hypertension, type 1 or type 2 diabetes mellitus, seizure disorder, renal disease, or liver disease), smoking, and drug or alcohol use. A control group of normotensive women aged 18–35 years with a singleton pregnancy delivered at term and no medical or obstetric complications was also recruited. All participants included in this study were from a low socioeconomic group according to Kuppuswamy's socioeconomic scale [19]. The present study was approved by the institutional ethics committee of Bharati Vidyapeeth Medical College, Bharati Vidyapeeth University; written consent was provided by all participants.

A blood pressure of greater than 140/90 mm Hg and concomitant proteinuria (>1+ on a dipstick test or 300 mg in 24 hours) was considered indicative of pre-eclampsia. Diagnosis was confirmed by repeated blood pressure measurements at 6-hour intervals. Women with pre-eclampsia were subdivided into two subgroups: term (delivery at ≥37 weeks) and preterm (delivery at <37 weeks). Gestational age was estimated from the last menstrual period and confirmed by ultrasonography. Birth weight, length, head circumference, and chest circumference were recorded within 30 minutes of delivery.

Fresh placental samples were collected immediately after delivery and the fetal membrane removed. The placental sampling sites used in the present study were based on those reported in previous studies [12,20]. Specimens were collected from the central maternal, central fetal, peripheral maternal, and peripheral fetal regions of the placenta. The site where the umbilical cord joined was considered the central region, whereas the peripheral region was defined as the site furthest from the cord insertion site. For both the central and peripheral regions, small pieces of placenta (approximately 1 × 1 cm) were cut from the basal plate (representing the maternal side) and from the chorionic plate (representing the fetal side). To avoid areas rich in intervillous fibrin, peripheral region samples were taken 1 cm away from the lateral edge of the placental disk. The tissues were rinsed in a working-strength solution of phosphate-buffered saline (1X) to remove maternal and fetal blood and then stored at –80 °C until analyzed.

Tissue samples weighing 0.2 g were homogenized with chilled phosphate-buffered saline and centrifuged at 10 000 revolutions per minute at 4 °C for 20 minutes to separate the cell membrane and supernatant fractions. The clear supernatant (homogenate) was transferred to a sterile vial. Total protein content of the homogenate was then estimated by the Lowry method.

The levels of MDA (expressed as μM/mg of total protein) were estimated using the BIOXYTECH MDA-586 spectrophotometric assay (Oxis International, Foster City, CA, USA). The activities of catalase (expressed as U/mg of total protein) and cellular GPx (GPx1) (expressed as mU/mg of total protein) were measured using the BIOXYTECH Catalase-520 and BIOXYTECH GPx-340 spectrophotometric assays, respectively. Samples were analyzed in duplicate and all assays were conducted according to the manufacturer's instructions. The detection limit (sensitivity) was 0.0801 μM for MDA and 1.71 U/mL for catalase. The assay range for GPx was 5.6–24.0 mU/mL.

The data were analyzed using SPSS/PC+ version 20 (IBM, Armonk, NY, USA). Values were generally reported as the mean ± standard deviation; however, the oxidative stress index (i.e. ratio of MDA to catalase activity and ratio of MDA to GPx activity) was calculated as the mean ± standard error. Normal distribution of the data was verified by testing for skewness and kurtosis. Any skewed variables were normalized by log transformation. Mean values derived from the four placental regions were compared using one-way analysis of variance and the post hoc least significant difference test. Mean between-group values of the different placental regions were compared using unadjusted independent sample *t* tests. $P < 0.05$ was considered statistically significant.

3. Results

A total of 60 participants were enrolled: 35 were normotensive, 11 had pre-eclampsia and delivered at term, and 14 had pre-eclampsia and delivered preterm. Maternal age was higher in the preterm pre-eclampsia group than in the other two groups ($P < 0.05$ for both) (Table 1). Body mass index was significantly higher in the term pre-eclampsia group than in the other groups ($P < 0.05$ for both) (Table 1). The systolic and diastolic blood pressures were higher in the term pre-eclampsia and preterm pre-eclampsia groups than in the normotensive group ($P < 0.01$ for both) (Table 1). Additionally, diastolic blood pressure was significantly higher in the preterm pre-eclampsia group than in the term pre-eclampsia group ($P < 0.05$) (Table 1). Birth weight, length, chest circumference, and head circumference were lower in the preterm pre-eclampsia group than in the normotensive group, and in the preterm group versus the term group ($P < 0.01$ for both) (Table 1).

In the normotensive and term pre-eclampsia groups, mean MDA levels were similar across all four regions of the placenta (Fig. 1). However, in the preterm pre-eclampsia group, the mean MDA levels were higher in the central maternal region than in the central fetal region (409.60 μM/mg of total protein vs 233.24 μM/mg of total protein; $P = 0.023$). The MDA levels were also higher in all four regions in the term

Table 1
Maternal and neonatal characteristics.[a]

Characteristic	Normotensive (n = 35)	Term pre-eclampsia (n = 11)	Preterm pre-eclampsia (n = 14)
Age, y	23.9 ± 2.8	25.2 ± 3.5	28.4 ± 4.5[b,c]
BMI	23.5 ± 3.5	30.6 ± 5.8[b]	26.4 ± 5.9[c]
Length of pregnancy, wk	39.0 ± 1.1	38.3 ± 1.3	34.4 ± 1.9[b,d]
Length of education, y	10.2 ± 4.4	11.4 ± 3.7	10.8 ± 4.0
Systolic blood pressure, mm Hg	119.6 ± 8.7	150.9 ± 19.7[b]	157.6 ± 22.7[b]
Diastolic blood pressure, mm Hg	77.5 ± 4.9	94.36 ± 9.2[b]	102.1 ± 12.5 [b,c]
Birth weight,	2900 ± 300	2800 ± 400	1800 ± 500[b,d]
Birth length, cm	49.1 ± 3.2	47.8 ± 2.9	42.8 ± 3.5[b,d]
Birth head circumference, cm	33.8 ± 1.4	34.3 ± 1.6	30.8 ± 2.6[b,d]
Birth chest circumference, cm	32.7 ± 1.4	32.0 ± 2.0	26.7 ± 3.7[b,d]
Placenta weight, g	516.29 ± 78.03	485.7 ± 57.98	335.83 ± 51.34[b,d]
Parity			
Nulliparous	15 (43)	8 (73)	2(14)
Multiparous	20 (57)	3 (27)	12 (86)
Mode of delivery			
Vaginal	31 (89)	6 (55)	3 (21)
Cesarean	4 (11)	5 (45)	11 (79)

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).
[a] Values given as mean ± SD or number (percentage).
[b] $P < 0.01$ vs normotensive.
[c] $P < 0.05$ vs term pre-eclampsia.
[d] $P < 0.01$ vs term pre-eclampsia.

Please cite this article as: Sahay AS, et al, Regional differences in the placental levels of oxidative stress markers in pre-eclampsia, Int J Gynecol Obstet (2015), http://dx.doi.org/10.1016/j.ijgo.2015.03.001