Assessment of Placental Oxidative Stress in Pre-Eclampsia

Das Brahmarshi · Saha-Roy Santa ·
Das Gupta Anindya · Lahiri Tapan Kumar ·
Das Harendra Nath

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Abstract

Objective To study oxidative stress in placental tissue as well as in serum in pre-eclamptic women.

Methods Fifty pre-eclamptic cases and fifty normal pregnant women were selected in the study. Thio barbituric acid reacting substances (TBARS) was measured as oxidative stress marker and superoxide dismutase (SOD) and GSH (reduced glutathione) were measured for assessment of antioxidant status in placental tissue extract and serum.

Results TBARS and SOD activity were increased significantly ($P < 0.001$) in both placental homogenate and

serum in pre-eclamptic women. Level of GSH was not altered much.

Conclusion Placental oxidative stress can be assessed by measuring serum oxidative stress markers and this may help in prevention of further progress of this condition.

Keywords Preeclampsia · Placental oxidative stress · TBARS

Introduction

Pre-eclampsia is a pregnancy related disorder, where there is development of hypertension and proteinuria with or without edema in a normotensive and non-proteinuric women after 20 weeks of gestation. The changes usually subside after delivery of placenta. The exact cause of these maternal changes in relation to pregnancy is not clear. There is generalized maternal vascular endothelial dysfunction and leukocyte activation [1–3]. A number of factors, generated in the placental tissue in pre-eclamptic women, pass to maternal system through fetoplacental circulation leading to these maternal changes. The factors include cytokines, pressor substances and oxidative stress factors. A number of observations point that there is excess generation of reactive oxygen species (ROS) in these placental tissue due to some anomaly in spiral arteries [4]. Increased ROS generation produces oxidative stress factors in placental tissue, which pass to maternal circulation to cause the changes [5]. In the present study, TBARS, SOD

Das B., Postgraduate Student ·
Das Gupta A., Associate Professor
Department of Biochemistry, Burdwan Medical College,
Burdwan, West Bengal, India

Saha-Roy S., Assistant Professor · Das H. N., Professor
Department of Biochemistry, Bangur Institute of Neurosciences,
Kolkata, West Bengal, India

Lahiri T. K., Professor
Department of Gynecology and Obstetrics, Calcutta Medical College,
Kolkata, West Bengal, India

Lahiri T. K. (✉), Professor
23/1, Sheikh Para Lane, P.O. Santragachi, Howrah 711104,
West Bengal, India
e-mail: lahiritapan@yahoo.co.in

and GSH were measured in placental tissue as well as in serum of pre-eclamptic and normal pregnant women to assess oxidative stress induced lipid peroxidation and maternal anti oxidant status.

Material and methods

Fifty pre-eclamptic cases and fifty normal pregnant women as control, attending the out patient department or admitted in the Department of Obstetrics and Gynaecology of Burdwan Medical College, Burdwan, WB were selected for this study. Necessary permission was taken from ethical committee.

Inclusion Criteria of Cases

- (1) Blood pressure: $\geq 140/90$ mmHg
- (2) Proteinuria: >300 mg/day
- (3) Above 20 weeks gestation

Exclusion Criteria of Cases

- (1) History of hypertension
- (2) History of kidney diseases

Placental tissue and clotted blood were collected from the same individual taking usual precautions.

Preparation of Placental Homogenate

Placental tissue was collected from freshly delivered placenta and transferred to laboratory from labour room or OT in a beaker on ice. The samples are homogenized as follows. Six grams of placental tissue were taken after thorough wash with ice-cold saline and properly homogenized with 6 ml of sodium pyrophosphate buffer in a homogenate tube on ice. The homogenate was centrifuged at 15,000 rpm for 10 min in a cold centrifuge machine. Supernatant was taken as sample for investigation. The parameters were analyzed maintaining the cold chain. The values of parameters are expressed per milligram or gram of tissue protein.

Measurement of Tissue Protein

Tissue proteins were measured by Lowry's method.

Measurement of Tissue TBARS

TBARS was measured according to method of Dahle [6]. To 0.5 ml of freshly prepared tissue extract, 2.5 ml of trichloroacetic acid was added and the tube was allowed to

stand for 10 min at room temperature. Then 2.5 ml of sulphuric acid was added and stirred thoroughly. 3.5 ml of TBA (thio-barbituric acid) reagent was added to this. Heating the mixture in boiling water bath for 30 minutes carried out the coupling of lipid-peroxide with TBA. It was then cooled in water. Then 4.0 ml. of n-butanol was added and chromogen was extracted to organic phase by vigorous shaking and vortexing.

Separation of the organic phase was facilitated by centrifugation at 3,000 rpm for 10 min. The supernatant organic phase was pipetted to a clean test tube. Its absorbance was determined at 532 nm wavelength by spectrophotometer (spectronic-21). n-Butanol was used as blank to assure zero reading. The optical density was noted and level of TBARS was calculated from standard curve. TBARS values were expressed in nmol/mg of tissue protein.

Measurement of Tissue SOD

SOD was estimated by method of Kakkar [7]. 3.35 ml of double distilled water, 50 μ l of tissue extract, 1.2 ml of sodium pyrophosphate buffer (pH = 8.3), 0.1 ml of PMS and 0.3 ml of NBT were mixed in a clean and dry centrifuge tube. 0.2 ml of NADH solution was added to it to initiate the reaction. After incubation at 39 °C for 90 s, the reaction was terminated by adding 1 ml of glacial acetic acid. 4 ml of n-butanol was then added and mixed vigorously by vortexing. Then the mixture was centrifuged at 4,000 rpm for 10 min and the absorbance of the upper butanol layer was measured at 560 nm. For comparison, corresponding blank was prepared in the same way except the addition of tissue extract. SOD values were expressed in U/mg of tissue protein.

Measurement of Tissue GSH

GSH was estimated by method of Dacie and Lewis. To 0.2 ml of tissue extract and 3 ml of precipitating solutions were mixed and then the solution was allowed to stand for 5 min. The mixture was filtered through a single thickness Whatman No-42 filter paper. Then 1 ml of clear filtrate and 4 ml of freshly made Na_2HPO_4 solution were mixed. The absorbance was recorded at 412 nm (A_1). 0.5 ml of the DTNB reagent was then added with the previous mixture. The colour developed rapidly and remained stable for about 10 min. The absorbance was recorded at 412 nm (A_2) using a spectrophotometer. A reagent blank was made using normal saline. GSH values in placental tissue were expressed in $\mu\text{g}/\text{mg}$ of tissue protein.

Measurement of Serum TBARS, SOD and GSH

Measurement of serum TBARS, SOD and GSH was done by same procedure as with tissue parameters, but with serum as sample.

Measurement of Urinary Protein

Urinary protein was measured by turbidimetric method [4].

The data obtained from the above tests were analyzed for difference between means. Independent *t* test was used to determine whether difference between means were significant. All these statistical analysis were carried out using SPSS software.

Results and Discussion

Result shows that there are significant increase of both TBARS ($P < 0.001$) and SOD activity ($P < 0.001$) in pre-eclamptic placental tissue as compared to control (Table 1). Serum TBARS and SOD activity in pre-eclamptic women are also observed to be raised in comparison to that of control sera (Table 2). TBARS is a group of compounds, formed due to lipid peroxidation, which occurs by the effect of hydroxyl free radicals on polyunsaturated fatty acids. Increased tissue TBARS formation is indicative of increased free radical generation. From the results of placental TBARS estimation it is clear that there is increased generation of ROS in the placental tissue due to some reason. The superoxide generation is usually the initiation of formation of ROS due to incomplete reduction of molecular oxygen. Increased SOD activity, observed in this placental tissue might be due to rise in superoxide generation. Changes in maternal serum TBARS and SOD might be due to transfer of these substances through feto-placental circulation leading to generation of maternal oxidative stress. Serum maternal oxidative stress is one of the hypotheses of development of pre-eclampsia [3]. Placental tissue GSH level in pre-eclamptic placenta was not altered in respect to control (Table 1). Similar pattern was also reflected in serum (Table 2). The placental tissue is probably unable to

upregulate GSH synthesis in proportion to SOD induction. This results in accumulation of hydrogen peroxide leading to increased generation of hydroxyl free radicals causing rise of lipid peroxidation and TBARS generation.

In pre-eclampsia, an otherwise normotensive individual develops hypertension after 20 weeks of gestation. She develops pitting oedema and passes albumin through urine. The cause of these changes is described due to generalized maternal endothelial cell dysfunction and leucocyte activation [1–3]. Endothelial cell dysfunction is characterized by altered state of endothelial cell differentiation in response to sub-lethal injury or cytotoxic stimulation. The endothelial cell becomes larger and may contain lipid droplets [8]. There is increased generalized vasoconstriction leading to maternal hypertension and reduced blood flow to organs and tissues including kidney, uterus etc. On the basis of a number of experimental observations a number of theories have been proposed as the cause of these changes. It has been proposed that a number of feto-placental units enter the maternal circulation and initiate the maternal patho-physiologic changes of pre-eclampsia [1, 2]. Lipid peroxidation products have been marked as one group of such compounds. The cause of this increased rate of lipid peroxidation due to rise in superoxide ion generation in placental tissue has been proved to be due to activation of xanthine oxidase and NADPH oxidase [9, 10].

In a case of normal pregnancy, there is conversion of spiral arteries from its highly tortuous thickened wall vessels to flaccid sinusoidal condits of low resistance. In pre-eclampsia the muscular coat of spiral arteries are retained [4]. They are susceptible to maternal humoral and neuronal constrictor influence. This affects the placental tissue causing hypoxia and reoxygenation. Hypoxia/reoxygenation is a potent stimulator of xanthine oxidase and NADPH oxidase that increase synthesis of superoxide ion generation [11]. Increased lipid peroxidation occurs when

Table 1 Comparison of means of parameter for oxidative stress in placental tissue of pre-eclamptic (case) and normal pregnant women (control)

Parameters	Pre-eclamptic women (case)	Normal pregnant women (control)	<i>P</i> value
Tissue TBAARS concentration (nmol/mg of tissue protein) [mean $\pm$ SD]	65.62 $\pm$ 4.33	37.07 $\pm$ 1.6	<0.001
Tissue SOD activity (U/mg of tissue protein) [mean $\pm$ SD]	13.84 $\pm$ 1.05	9.9 $\pm$ 0.76	<0.001
Tissue GSH concentration (μ g/g of tissue protein) [mean $\pm$ SD]	0.93 $\pm$ 0.09	0.92 $\pm$ 0.17	0.844

Table 2 Comparison of means of parameter for oxidative stress in serum of pre-eclamptic (case) and normal pregnant women (control)

Parameters	Pre-eclamptic women (case)	Normal pregnant women (control)	<i>P</i> value
Serum TBARS (nmol/ml) [mean $\pm$ SD]	9.37 $\pm$ 1.07	4.89 $\pm$ 0.85	<0.001
Serum SOD (Unit/ml) [mean $\pm$ SD]	12.51 $\pm$ 1.18	6.42 $\pm$ 1.11	<0.001
Serum GSH (μ g/l) [mean $\pm$ SD]	0.43 $\pm$ 0.04	0.43 $\pm$ 0.08	0.733

body's antioxidant status fails to overcome this, and the situation is termed as oxidative stress. The oxidative stress products are released in maternal circulation producing the maternal changes. Pre-eclampsia, thus is a result of multiple phenomena working together [12].

From our study it is also clear that placental tissue of the pre-eclamptic women is in significant oxidative stress. The stress is equally reflected in the maternal serum. Therefore serum TBARS estimation may indicate placental oxidative stress status. Although it is not clear whether placental oxidative stress begins long before the pre-eclamptic symptoms appear, if it is so, then routine assay of serum TBARS may be of predictive value for development of pre-eclampsia. Detailed study may be of help in this regard.

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