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Neurotrophin levels in different regions of the placenta and their association with birth outcome and blood pressure

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ABSTRACT

Introduction: Our recent study in preeclampsia indicates differential oxidative stress in various regions of the placenta. Oxidative stress is known to influence neurotrophin levels. We therefore hypothesize that placental regional differences in oxidative stress will also lead to differences in neurotrophin levels.
Methods: The current study examines the levels of neurotrophins, brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in different regions of the placenta in 50 normotensive control women and 47 women with preeclampsia (21 delivering at term and 26 delivering preterm). Placentae were collected from four different regions: central maternal (CM), central fetal (CF), peripheral maternal (PM) and peripheral fetal (PF).
Results: BDNF levels were higher in CF region as compared to CM ($p < 0.01$), PM ($p < 0.01$) and PF ($p < 0.05$) regions of the placenta in the control group. There was no regional change in NGF levels in any of the groups. Analysis between groups indicated higher NGF levels in CM ($p < 0.01$), PM ($p < 0.05$) and PF ($p < 0.01$) regions of preterm preeclampsia group as compared to control. Negative association of NGF levels in CM, CF and PM regions with baby weight and in CF, PM and PF regions with baby length was observed. NGF levels in all four regions were positively associated with systolic blood pressure.
Discussion: Our data indicates regional differences in levels of BDNF only in normotensive control but not in preeclampsia group. Higher NGF levels in preterm preeclampsia may be a response to increased oxidative stress. This may have implications for altered placental development in preeclampsia.

1. Introduction

Preeclampsia, characterized by increased blood pressure and proteinuria after 20 weeks of gestation is one of the leading gestational hypertensive disorders with an unclear etiology [1]. It has been suggested that the altered placental development plays a central role in the pathogenesis of preeclampsia [2]. The placenta in preeclampsia is known to be affected with increased oxidative stress [3]. Defective trophoblast invasion in preeclampsia results in intermittent arterial blood flow creating a hypoxic environment which leads to oxidative stress [4]. Oxidative stress and inflammation are known to be closely related [5] and have been proposed to play a major role in the pathogenesis of preeclampsia [6,7].

Studies suggest that different regions of the placenta vary in the architecture, oxidative stress indices and oxygen availability [8–10] and are associated with pregnancy outcome [10]. Oxidative stress has been shown to regulate the expression of neurotrophins [11,12] which are known to influence the process of angiogenesis [13].

Neurotrophins like brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are reported to be present in the placenta [14,15]. BDNF exerts its effect by activating the high-affinity tyrosine kinase B (Trk B) receptor and also the low-affinity coreceptor p75 [16] while NGF binds to the p75 neurotrophin receptor (p75NTR) and tropomyosin related kinase A (TrkA) [17]. Studies suggest that BDNF/TrkB signaling system plays an important role during implantation, ensuing placental development and fetal growth by increasing trophoblast cell growth and survival [18]. A recent study suggests that both BDNF and its receptor TrkB may play a role in regulating glucose levels during pregnancy [19]. NGF mRNA expression has been shown in the trophoblast, amnion/chorion and maternal decidua in early gestation as well as at term [20]. Further, a recent study also suggests that for a healthy pregnancy an optimal expression of NGF at the

Abbreviations: CM, Central Maternal; CF, Central Fetal; PM, Peripheral Maternal; PF, Peripheral Fetal.

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fetal-maternal interface is essential [21].

These neurotrophins influence the process of angiogenesis as they exert powerful angiogenic effect [13]. Regional differences in expression of angiogenic factors has been reported with enhanced vascular endothelial growth factor (VEGF) expression in the subchorionic lateral border compared to medial basal site [22]. It has been suggested that neurotrophins influence the development and maturation of the feto-placental unit during gestation and affect fetal growth trajectories [23].

We have recently reported differentially increased oxidative stress in four different regions of placenta (central maternal (CM), central fetal (CF), peripheral maternal (PM) and peripheral fetal (PF)) in women with preeclampsia as compared to normotensive women. In addition, we have also reported increased oxidative stress in the central maternal region as compared to central fetal region in the women with preeclampsia delivering preterm [24].

In view of this, we hypothesize that placental regional differences in oxidative stress will lead to differences in neurotrophin levels in different regions of the placenta. The current study examines the levels of neurotrophins (BDNF and NGF) in different regions of the placenta in normotensive women and women with preeclampsia delivering both at term as well as those delivering preterm. Further, their association with birth outcome and blood pressure was also examined.

2. Methods

2.1. Subjects

This cross sectional study was conducted at the Department of Obstetrics and Gynaecology, Bharati Hospital, Pune. The present study was approved by the Bharati Vidyapeeth Medical College Institutional Ethical Committee and a written consent was taken from each subject. A total of 97 pregnant women (50 normotensive control) and (47 women with preeclampsia) were included in this study. Amongst the 47 women with preeclampsia, 21 women delivered at term ($\geq$37 weeks) and 26 delivered preterm (<37 weeks). The power of the current study was calculated based on our previous study which examined region specific changes in the levels of placental malondialdehyde (MDA) levels ($p < 0.05$) between normotensive women ($n = 35$) and women with preeclampsia delivering both at term and preterm ($n = 25$) [24]. For the current study the sample size was 50 normotensive control women, 21 women with preeclampsia delivering at term and 26 women with preeclampsia delivering preterm were included. This sample size gives a power of 85% with 0.05 type I error probability and 2 control subjects per case.

Singleton pregnant women with age group of 18–35 years were included in this study. Normotensive control women included those women with no obstetrical or medical complications. Preeclampsia was defined by systolic and diastolic blood pressures greater than 140 and 90 mm Hg, respectively, with presence of proteinuria (>1 + on a dipstick test). Preeclampsia was confirmed by repeated recording of the blood pressure with an interval of 6 h. The exclusion criteria included those women with any other pregnancy complications, such as chronic hypertension, type I or type II diabetes mellitus, multiple gestations and seizure disorder. Pregnant women who smoked as well as those with alcohol or drug abuse were also excluded from the study.

2.2. Sample collection and processing

Fresh placentae were obtained from normal and preeclampsia pregnancies immediately after delivery. From the chorionic plate (representing fetal side) the membranes were removed thus the fetal samples did not include the membranes, whereas the maternal samples from the basal plate (representing maternal side) included the decidua. Detailed method of sample collection has been described in our earlier study [24]. Briefly, placental tissue samples were collected from four different regions in the placenta: i.e i. Central maternal (CM), ii. Central fetal (CF), iii. Peripheral maternal (PM) and iv. Peripheral fetal (PF). Central region was considered as the site where the cord was inserted and the site farthest from cord insertion was considered as peripheral region. Small pieces (approx 1 cm [length] * 1 cm [width]) were cut from the basal plate (representing maternal side) and from the chorionic plate (representing fetal side) from both the central and peripheral regions. The placental tissues were rinsed in 1X PBS and then stored at −80 °C until analyzed.

2.3. Birth outcome measures

Birth outcome measures like baby weight and length were recorded as described earlier [25].

2.4. Preparation of placental tissue homogenates

Placental tissues were homogenized in chilled PBS as described in our earlier study [24]. Total protein content of the lysate was then estimated by Lowry method [26].

2.5. NGF levels from different regions of the placenta

NGF levels were measured in placental supernatants using the NGF Emax Immuno Assay System (Promega) and the method has been reported by us earlier [27]. NGF concentrations are expressed as pg/mg of total protein.

2.6. BDNF levels from different regions of the placenta

BDNF levels were measured in placental supernatant using the BDNF Emax Immuno Assay System (Promega) and the method has been reported by us earlier [28]. BDNF concentrations are expressed as pg/mg of total protein.

2.7. Statistical analysis

All values are reported as mean ± standard error (S.E). The data were analyzed using the SPSS/PC + package (Version 20, Chicago, IL, USA). The data were checked for normal distribution by testing for skewness and kurtosis. Skewed variables were transformed to normality using the log to the base 10 transformation. Mean values of different regions in respective groups were compared using one way analysis of variance (ANOVA) and the post-hoc least significant difference (LSD) test. Mean values of different regions between groups were compared using unadjusted independent sample t-tests to identify statistically significant differences ($p < 0.05$). Correlation of placental neurotrophins (NGF and BDNF) with placental MDA levels was studied by Pearson correlation analysis. Correlation of placental neurotrophins (NGF and BDNF) with baby weight, baby length and maternal blood pressure was studied using partial correlation analysis after adjusting for gestation, age, body mass index (BMI) and group.

3. Result

3.1. Demographic characteristics of normotensive mothers and their neonates

Table 1 shows the maternal and neonatal demographic

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