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Maternal nerve growth factor levels during pregnancy in women with preeclampsia: A longitudinal study

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ABSTRACT

Preeclampsia (PE) is characterized by hypertension and proteinuria. Improper development of the placenta due to altered angiogenesis is the main culprit in PE. Nerve growth factor (NGF) is an angiogenic factor which is expressed and localized in the placenta. Our earlier cross sectional study has shown altered NGF levels at delivery in women with PE. However, there are no studies on NGF levels in PE early in pregnancy before manifestation of the disease. Thus, there is a need to examine the role of NGF in vascular development during different stages of gestation in PE. A longitudinal study was carried out where pregnant women were enrolled from two major hospitals from Pune, Bharati hospital and Gupte hospital. They were followed at three different time points [16–20 weeks (T1), 26–30 weeks (T2) and at delivery (T3)] during pregnancy and maternal blood at every time point and cord blood at delivery was collected and processed. This study included normotensive women ($n = 88$) and women with PE ($n = 48$). NGF levels were measured from maternal and cord plasma using the Emax Immuno Assay System (Promega). The data was analyzed using the SPSS/PC+ package (Version 20.0, Chicago, IL, USA). Maternal NGF levels did not change at all time points while cord NGF levels were higher ($p < 0.05$) in women with PE. Further, maternal NGF levels were negatively associated with blood pressure while cord NGF levels were positively associated with baby head circumference. Our data suggests that there may possibly be a compensatory role for NGF in the foeto-placental circulation in PE.

1. Introduction

Preeclampsia (PE), a pregnancy complication, characterized by hypertension and proteinuria, occurs after 20 weeks of gestation and is the major cause of maternal, fetal, infant mortality and morbidity, preterm birth and intrauterine growth restriction (IUGR) (Young et al., 2010; Duley, 2008; Winn et al., 2011; Backes et al., 2011). The etiology of preeclampsia is unknown, but studies have reported that this disorder appears to originate in placenta and is characterized by widespread maternal endothelial dysfunction (Petla et al., 2013).

Abnormal placentation and reduced blood flow in PE is a result of impaired invasion of maternal spiral arteries (Steegers et al., 2010; Myatt and Webster, 2009). In a normal pregnancy, development of the placenta is controlled by a plethora of growth factors, such as cytokines, neurotrophins like nerve growth factor (NGF) and angiogenic molecules controlling trophoblast motility, which are secreted from numerous trophoblast cells that regulates trophoblast invasiveness (Knöfler, 2010). Defective expression and activity of these molecules are potential candidates for triggering PE (Ong, 2004). NGF is expressed and is active in different parts of the placenta in early gestation and at term (Toti et al., 2006; Marvin et al., 2002). This expression may be differentially regulated in PE due to maldevelopment of placenta.

NGF plays an important role in the regulation of growth, differentiation and survival of neurons in both the central and peripheral nervous systems (Bibel and Barde, 2000). Although NGF has historically been implicated in several functions of the nervous system,

Abbreviations: PE, preeclampsia; BMI, body mass index; BP, blood pressure; DHA, docosahexaenoic acid; mAb, monoclonal antibody; NC, normotensive control; NGF, nerve growth factor; pAb, polyclonal antibody; Trk B, tyrosine kinase receptor B; VEGF, vascular endothelial growth factor.

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it can also regulate hypertension (Hennigan et al., 2009; Supowit et al., 2001) and elicit cardiovascular actions, including angiogenesis during fetal and neonatal stages (Meloni et al., 2010; Cabrera-Vásquez et al., 2009). Tometten et al. (2005) reported that NGF is mandatory for the success of pregnancy as it constitutes a functional link between the nervous, immune and endocrine systems and translating environmental or endocrine signals (Tometten et al., 2005). It has been reported that higher NGF levels in pregnancy which are progressive as trimester progresses in monkeys as compared to non pregnant monkeys (Neubert et al., 2014).

Further, our earlier cross-sectional study on women with PE has shown altered NGF levels as compared to normotensive women (Kilari et al., 2011). There are very few studies, which have studied the levels of NGF in different pregnancy complications like IUGR (Malamitsi-Puchner et al., 2007) but not in women with PE. Xia et al., (2011) have shown reduced NGF levels in amniotic fluid specimens obtained during amniocentesis or caesarean section. Although PE is characteristically diagnosed in the last third of pregnancy, it is apparent that most of these pathophysiological changes can be detected long before clinically evident disease (Roberts and Bell, 2013). Hence, there is a need to study the maternal and cord NGF levels across the pregnancy to know to study the mechanism and severity of PE.

Therefore, in order to better understand the role of NGF in pathology of PE, the present study for the first time examines maternal NGF levels across gestation along with cord NGF levels in women with PE and their associations with mother's blood pressure (BP) and baby's birth outcome.

2. Materials and methods

2.1. Study subjects

This study was a part of a large ongoing departmental study that recruited singleton pregnant women at 16–20 weeks of gestation and followed them until delivery. Women were categorized as having PE if there is a presence of high blood pressure and proteinuria. PE was defined by systolic and diastolic blood pressures of >140 mmHg and >90 mmHg respectively with the presence of proteinuria (>1+ or 300 mg per 24 h). Mercury sphygmomanometer was used to measure blood pressure and PE was diagnosed and confirmed by repeated readings of blood pressure. Proteinuria was measured on a dipstick test. This study included normotensive ($n = 88$) and women with PE ($n = 48$). Women who delivered at term (gestation $\geq$37 weeks) with normal baby birth weight ($\geq$2.5 kg), no medical or obstetrical complications throughout pregnancy were considered as normotensive controls. The details of number of samples at each time point are given as a flow chart (Fig 1). Women were excluded from the study if there was an evidence of other complications such as type I or type II diabetes mellitus, chronic hypertension, seizures, liver or renal disorders. All study participants neither consumed alcohol nor smoked. Maternal blood samples were collected at three time points i.e., at 16–20 weeks (T1), at 26–30 weeks (T2) and at the time of delivery (T3). Umbilical cord blood (mixture of both arterial and venous blood) (T4) and placental samples were also collected just after delivery of the baby. The umbilical cord blood was also collected just after delivery of the baby (T4). The detailed study design has been described by us earlier (Wadhwani et al., 2014; Sahay et al., 2015).

In this study, based on the gestation, preeclampsia group was divided as Term PE and Preterm PE since the degree and severity of pathology is more in early onset of preeclampsia and leads to preterm delivery. Neonatal measures like birth weight, length, head and chest circumferences were recorded. Birth weight was

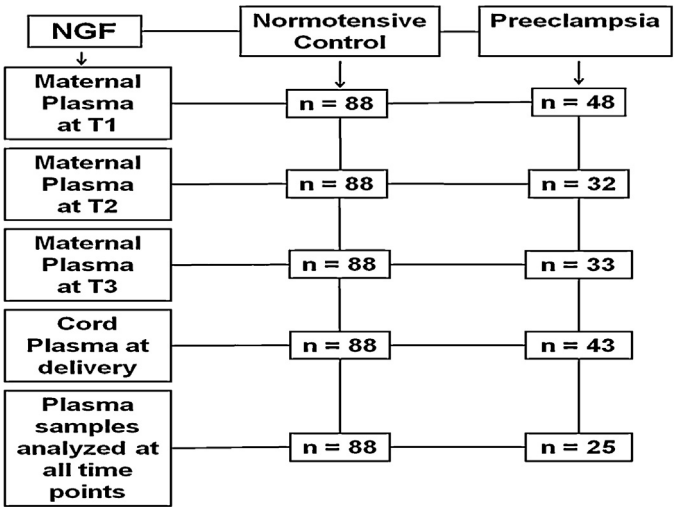

Fig. 1. Flowchart of number of samples analyzed for NGF.
T1—16–20 weeks of gestation; T2—26–30 weeks of gestation; T3—at delivery, NGF —Nerve Growth Factor; n—number.

recorded using a digital weighing scale with an accuracy of 10 g (Zeal medical private limited, India). The length was measured using a portable Infantometer. The head and chest circumferences were measured using fiber glass measuring tape placing around the head and lower chest respectively. The details of these procedures have been described in our earlier study (Sundrani et al., 2013).

2.2. Power of the study

There are no studies available in the literature on plasma NGF levels at various time points across gestation. Since this is the first longitudinal study of NGF in women with preeclampsia, the power of the current study was calculated based on our earlier cross sectional study on NGF levels in preeclampsia (Kilari et al., 2011). Based on results from this study, sample size for current study was calculated using Power and Sample Size Calculation software (version 3.0.43). In our earlier study, NGF levels in NC (316.3 $\pm$ 109.7 pg/ml) and PE (257.6 $\pm$ 122.85 pg/ml) group were normally distributed having a population standard deviation = 116.275. If the true difference in the means of NGF levels between PE and NC is 58.7, we would need to study 47 PE subjects and 94 NC subjects to be able to reject the null hypothesis that the population means of the preeclampsia and NC groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. In view of this, this study was undertaken on 88 NC who had complete data for all three time-points and the cord-blood sample. In case of preeclampsia, a total of 48 women were recruited out of which 25 women had complete data at all time points.

2.3. Sample collection, processing and storing

Maternal venous blood (10 ml) was collected at every time point into tubes containing EDTA (ethylenediamine tetraacetic acid). In addition, 10 ml cord blood was collected from the umbilical cord just after delivery. The blood was layered on density gradient Histopaque (Sigma–Aldrich, St. Louis, MO, USA) and spun at 1800 rpm for 35 min to separate the plasma and erythrocytes. Plasma aliquots were stored at −80 °C until further analysis and the method has been described earlier (Sundrani et al., 2013). Fresh placental tissues were obtained on a subsample from normal and preeclamptic pregnancies immediately after delivery. PE ($n = 24$) and control ($n = 22$) placental samples were collected and analyzed