NEUROTROPHINS AND OXIDATIVE STRESS IN PREECLAMPSIA

A thesis submitted to the

Bharati Vidyapeeth Deemed University, Pune

For award of degree of

DOCTOR OF PHILOSOPHY

In

Biotechnology

Under the

Faculty of Science

By

Ms. Vandita A. D'Souza

Under the guidance of

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April 2015

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Declaration by the Candidate

I hereby declare that the thesis entitled **"Neurotrophins and Oxidative Stress in Preeclampsia"** submitted by me to the Bharati Vidyapeeth University, Pune, for the degree of **Doctor of Philosophy (Ph.D.)** in Biotechnology under the Faculty of Science is an original piece of work carried out by me under the supervision of Dr. Sadhana R. Joshi. I further declare that it has not been submitted to this or any other university or institution for the award of any degree or diploma.

I also confirm that all the material which I have borrowed from other sources and incorporated in this thesis is duly acknowledged. If any material is not duly acknowledged and found incorporated in this thesis, it is entirely my responsibility. I am fully aware of the implications of any such act which might have been committed by me advertently or inadvertently.

Place: Pune

Date:

Ms. Vandita A. D'Souza

Research Student

Acknowledgement

Pursuing a Ph.D. has been a big challenge for me. It has included long working hours testing one's physical, mental and emotional capacity. Looking back to where I started, I have gained immensely from all the experiences obtained in the last few years. None of this would be possible without those who have helped, advised, opinionated, motivated and supported me. I would like to thank each and everyone who has made this thesis a reality.

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.....Vandita

THESIS AT A GLANCE

Neurotrophins and Oxidative Stress in Preeclampsia

Chapters	Publications	Conferences	Awards
1. Introduction and Genesis of the Thesis	Curr Med Chem. 2013; 20:4777-83 Impact factor: 3.715	1st David Barker memorial Symposium at 8th Annual Meet of Society for Natal Effects on Health in Adults, Lonavala, India, September, 2014	Registration And Travel Award Indian Council of Medical Research, New Delhi 8th World Congress on Developmental Origins of Health and Disease, Singapore, November 2013
2. Brain Derived Neurotrophic Factor Levels in Preeclampsia: A Cross Sectional Study	Reprod Sci. 2014; 21:230-5 Impact factor: 2.179	Recent Trends in Free Radical and Antioxidant Research & 13th Annual Meeting of the Society for Free Radical Research, Lonavala, India, January, 2014	
3. Gestation Dependent Changes in Brain Derived Neurotrophic Factor Levels in Preeclampsia	Int J Dev Neurosci. 2014; 37:36-40 Impact factor: 2.918	8th World Congress on Developmental Origins of Health and Disease. Singapore, September, 2013	
		44th National Conference of Nutrition Society of India, Tirupati, November, 2012	
4. Gestation Dependent Changes in Nerve Growth Factor Levels in Preeclampsia	Int J Dev Neurosci. 2015; "Submitted" Impact factor: 2.918	43rd National Conference of Nutrition Society of India, Hyderabad, November, 2011	
5. Gestation Dependent Changes in Oxidative Stress Markers and their Association with Neurotrophins in Preeclampsia	Scand J Clin Lab Invest Suppl. 2015; "Submitted" Impact factor: 2.009	7th World Congress on Developmental Origins of Health and Disease. Portland, Oregon USA, September, 2011	
		"Maternal Undernutrition and Developmental Origin of Health and Diseases" organized by National Institute of Nutrition (NIN), Hyderabad, India June, 2011	
Summary		42nd National Conference of Nutrition Society of India, Mumbai, India, November, 2010	
References			

List of Abbreviations

AA	Arachidonic Acid
ACOG	American Congress of Obstetrics and Gynaecology
ADHD	Attention Deficit Hyperactivity Disorder
ALA	Alpha Linolenic Acid
ANOVA	Analysis Of Variance
BDNF	Brain Derived Neurotrophic Factor
BMI	Body Mass Index
COMT	Catechol-O-Methyltransferase
COPD	Chronic Obstructive Pulmonary Disease
CREB	cAMP Response Element-Binding Protein
CTB	Cytotrophoblast
CVD	Cardiovascular Disease
DHA	Docosahexaenoic Acid
DOHaD	Developmental Origins of Health and Disease
eNOS	Endothelial Nitric Oxide Synthase
EPA	Eicosapentaenoic Acid
FLT-1	Fms-like Tyrosine Kinase-1
FOAD	Foetal Origins of Adult Disease
GPx	Glutathione Peroxidase
GR	Glutathione Reductase
GSH	Glutathione
GST	Glutathione Transferase
HELLP	Haemolysis, Elevated Liver Enzymes and Low Platelet
HIF	Hypoxia Inducible Factor
H_2O_2	Hydrogen Peroxide

IUGR	Intrauterine Growth Restriction
LA	Linoleic Acid
LBW	Low Birth Weight
LCPUFA	Long Chain Polyunsaturated Fatty Acids
MDA	Malondialdehyde
MS	Methionine Synthase
MTHFR	Methylene Tetrahydrofolate Reductase
NCD	Non-Communicable Disease
NGF	Nerve Growth Factor
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
PlGF	Placental Growth Factor
p75NTR	p75 Neurotrophin Receptor
Pro-NTs	Proneurotrophins
ROS	Reactive Oxygen Species
RT-qPCR	Real Time Quantitative Polymerase Chain Reaction
SAH	S-Adenosylhomocysteine
SAH-H	SAH Hydrolase
SAM	S-Adenosylmethionine
SOD	Superoxide Dismutase
SPSS	Statistical Package of Social Sciences
STB	Syncytiotrophoblast
Trk	Tropomyosin-related Receptor Kinase
VEGF	Vascular Endothelial Growth Factor

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CHAPTER 1

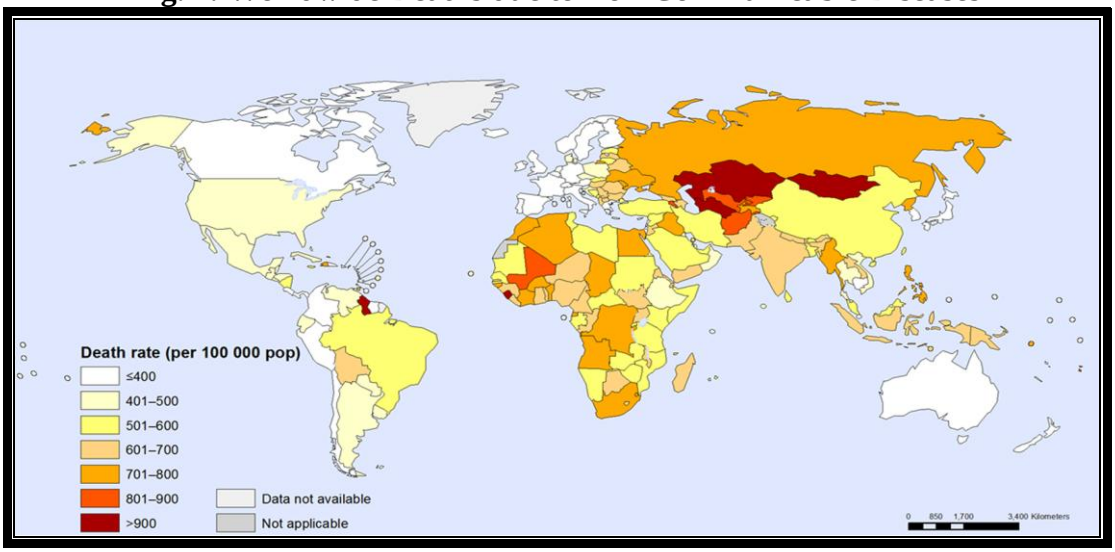
Introduction and Genesis of the Thesis

1.1 Link between Adverse Pregnancy Outcomes and Non-Communicable Diseases (NCDs)

1.1.1 Global Scenario of NCDs

Non-Communicable Diseases (NCDs) include cardiovascular diseases (CVDs), diabetes, cancer and mental disorders (WHO, 2013). Reports indicate that they are responsible for about two third of deaths worldwide (Ezzati and Riboli, 2012). According to the assessment of the World Health Organization (WHO), current global mortality from NCDs remains unacceptably high and is continuously increasing (WHO, 2014; Hanson and Gluckman, 2011) (Fig.1). Among NCDs, CVDs are the most prevalent and leading cause of death (Turk-Adawi *et al*., 2014) while cerebrovascular diseases are the second most common cause of mortality and are responsible for 6.15 million deaths (10.8% of all deaths) worldwide (Mehndiratta *et al*., 2013). Morbidity associated with NCDs threatens the health of populations and economies (Matheson *et al*., 2013) and hence there is an urgent need for preventive strategies.

Fig. 1: Worldwide Deaths due to Non-Communicable Diseases

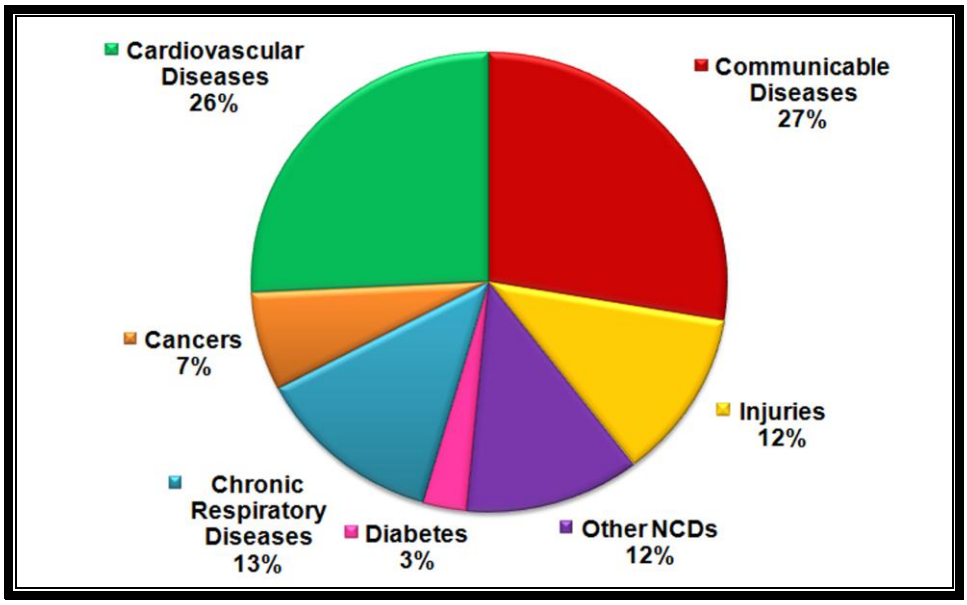


Source: WHO Map Production; Health Statistics and Information Systems (HSI); WHO 2014. http://gamapserver.who.int/mapLibrary/Files/Maps/Global_NCD_deaths_2012.png

1.1.2 Indian Scenario of NCDs

NCDs are reported to be responsible for 80% of deaths occurring in low and middle income countries like India (Roura and Arulkumaran, 2014) (Fig. 2). Among the emerging economies, India with the world's second largest population has the highest number of people with CVDs (Madan *et al*., 2014). The population in India is projected to have over 1 million strokes per year (Mehndiratta *et al*., 2013).

Fig. 2: India's Disease Profile



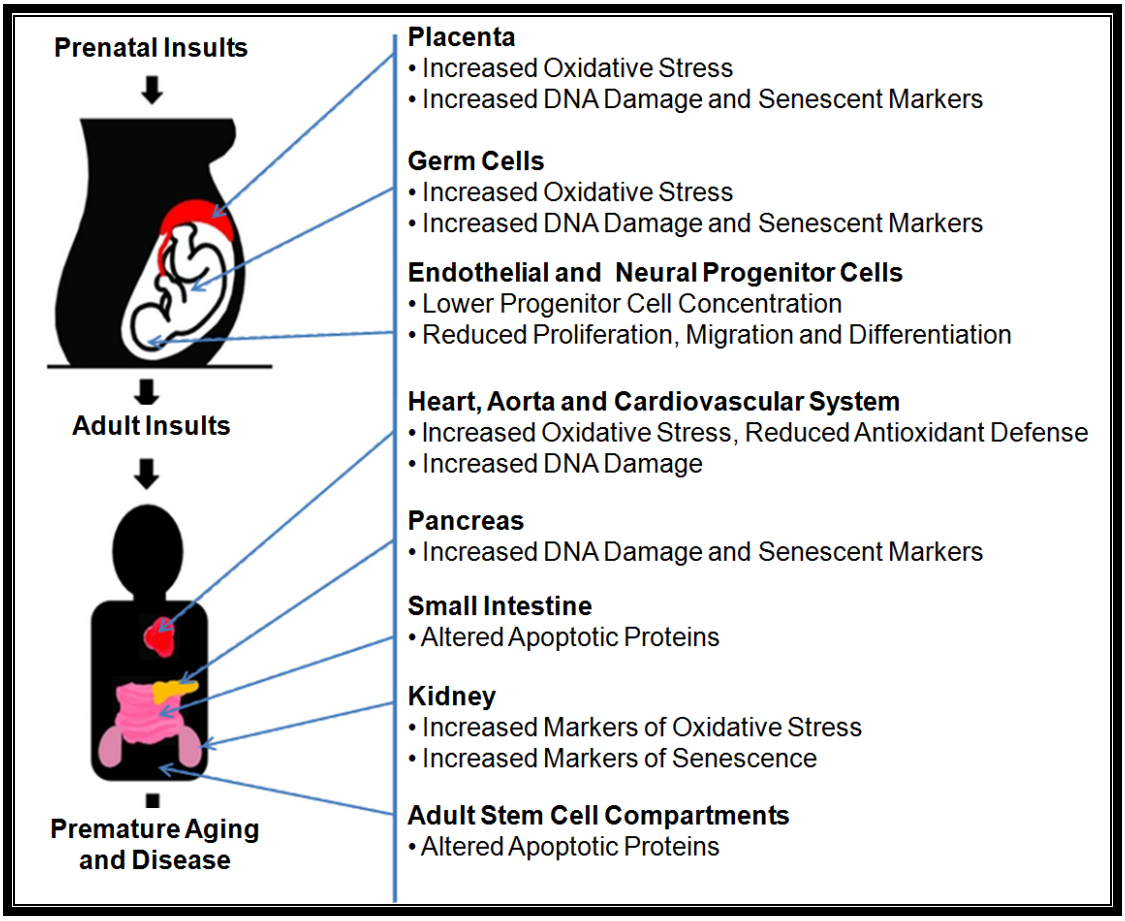
Source: WHO – NCD Country Profiles 2014
http://www.who.int/nmh/countries/ind_en.pdf?ua=1

1.1.3 Early Life Insults and Risk for NCDs

Risk factors for NCDs broadly include genetics, adult lifestyle, behavioral factors and early life exposures (Sears and Genuis, 2011; Burdge and Lillycrop, 2010). Research indicates that early development (*in utero* and early postnatal life) is susceptible to disruption by nutritional and environmental chemical exposures, with potentially adverse consequences for health later in life (Barouki *et al*., 2012; Hallows *et al*., 2012) (Fig. 3). Cues for plasticity operate particularly during early development

and via epigenetic mechanisms induced by environmental insults may affect a single organ or system and induce changes in the mature phenotype (Gluckman *et al*., 2007).

Fig. 3: Early Life Insults and Risk of Adult Diseases



Source: Hallows et al., J Pregnancy, 2012; 2012:638476.

Therefore, WHO suggests a need to focus efforts on improving maternal health to combat the growing burden of NCDs (WHO, 2013). The concept that the risk of developing NCDs in later life may be related to environmental exposures during the developmental period is known as 'Developmental Origins of Health and Disease' (DOHaD) (Langley-Evans, 2006).

1.2 Developmental Origins of Health and Disease

Studies by Late Prof. David Barker, Southampton University, UK and his colleagues during the late 1980s suggest that the incidence of adult diseases such as

stroke, type 2 diabetes and dyslipidaemia may be linked to *in utero* development and was initially referred to as the foetal origins of adult disease (FOAD) (Sinclair *et al*., 2007) (Fig. 4). This concept also referred to as 'Barker's hypothesis' emerged 25 years ago from epidemiological studies of birth and death records that revealed an association between birth weight and rates of adult death from ischemic heart disease (Wadhwa *et al*., 2009). Later the FOAD theory was referred to as the DOHaD to also include early postnatal exposures (Smith, 2012).

Fig. 4: Developmental Origins of Health and Disease by Late Prof. David Barker

Source: Journal of Developmental Origins of Health and Disease
http://journals.cambridge.org/action/displayJournal?jid=DOH
http://www.southampton.ac.uk/medicine/research/dohad.page
http://www.southampton.ac.uk/mediacentre/news/2013/sep/13_160.shtml

Research till date also shows that low birth weight, indicative of poor foetal growth is found to be associated with an increased risk of NCDs in later life (Alexander *et al*., 2014). The DOHaD hypothesis highlights the relation between the poor nutritional state during the periconceptional, embryonic, foetal and early infant phases and the subsequent metabolic disorders in later life (Fukuoka *et al*., 2014; Yan and Yang, 2014; Wadhwa *et al*, 2009; Sinclair and Singh, 2007). The DOHaD theory provides new insights into the molecular pathogenesis of NCDs.

Nutritional stress, multiple pregnancy, environmental stress, gynaecological immaturity and maternal or foetal genotype are the factors influencing developmental programming (Reynold *et al*., 2010). Among them quality or quantity of nutrient provision in pregnancy and/or lactation has a major impact on tissue development and function and can promote both disease susceptibility and resistance (Langley-Evans *et al*., 2006).

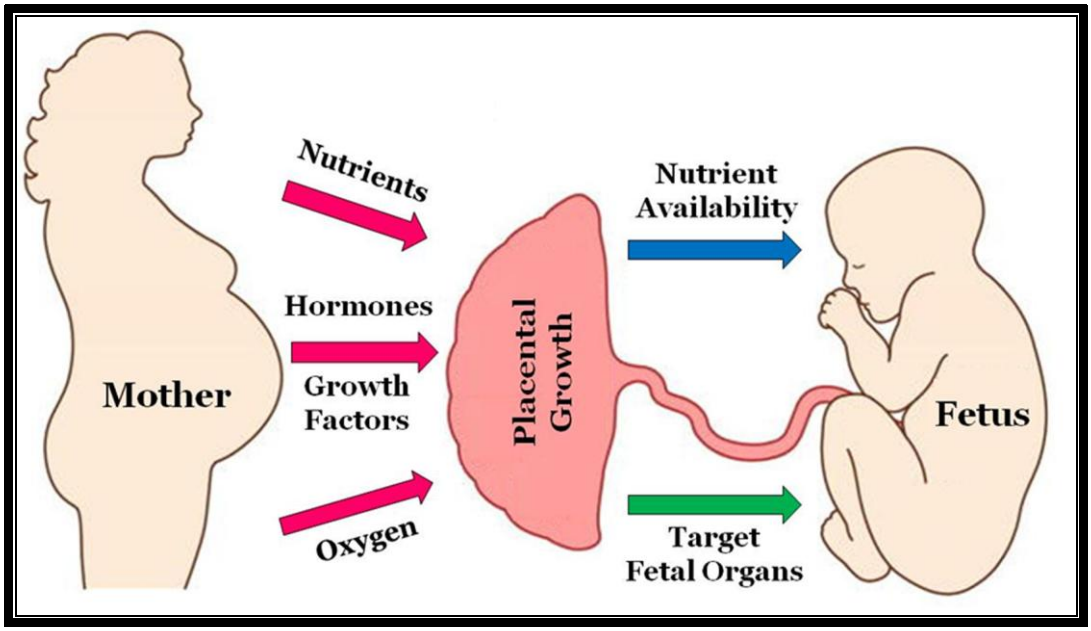
It is now widely accepted that early-life nutritional exposures affect disease development through epigenetic processes (Fall, 2013). Epigenetics is defined as the study of the inheritance (between cells and/or organisms) of traits (gene expression or phenotypes) without changes to the underlying deoxyribonucleic acid (DNA) sequence (Loi *et al*., 2013). Recent studies have now indicated that there are important links between pregnancy complications and epigenetic regulation in the placenta (Lesseur *et al*., 2014; Maccani and Marsit, 2009). A disturbance in epigenetic regulation of genes can lead to abnormal placental development and function with possible consequences for maternal morbidity, foetal development and disease susceptibility in later life (Nelissen *et al*., 2011).

1.2.1 The Placenta: A Programming Agent

The placenta is a specialized pregnancy specific organ that regulates metabolic processes between the mother and her developing foetus (Herrera *et al*., 2014; Gude *et al.*, 2004) (Fig. 5). Placental development is influenced by several growth factors, cytokines and transcription factors (Murthi, 2014). The placenta is involved in embryo implantation, transport of nutrients, elimination of metabolic waste products and endocrine activity (Giaginis *et al.*, 2012). Several mechanisms account for

transfer of substances across the placenta, including passive diffusion, facilitated diffusion and active transport (Hill and Longo, 1980).

Fig. 5: Transfer of Metabolites from Mother to Foetus via the Placenta

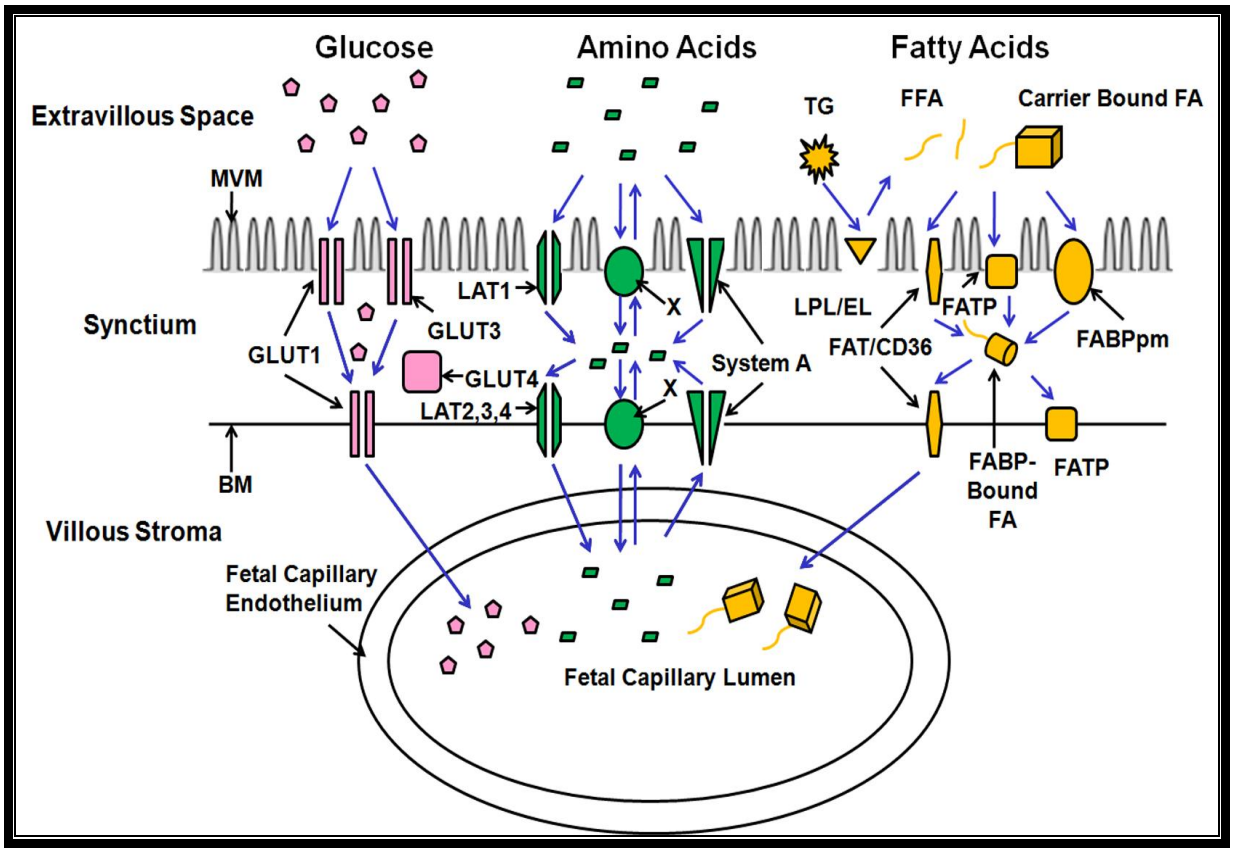


Source: Modified from Jansson et al., Placenta, 2012; 33:e23-e29

1.2.2 Nutrient Transfer to Foetus via Placenta

It is well known that the maternal nutritional state before and during very early pregnancy determines foetal health and survival (Bloomfield *et al.*, 2013). Trophoblast nutrient sensing signalling pathways in the placenta regulate cell metabolism suggesting the key role of placental nutrient uptake and transfer in foetal growth and health programming (Larqué *et al.*, 2013). This nutrient transfer from mother to foetus is maintained by expression of classic sugar, amino acid and fatty acid transporters at the trophoblast microvillous and basal membranes (Carter, 2012) (Fig. 6). Both the mother and child are vulnerable to changes in dietary supply, especially of those nutrients that are marginal under normal circumstances (Gambling and McArdle, 2004). Recent reports indicate that several biological and physiological mechanisms related to nutritive requirements together with their transfer and utilization across the placenta are still poorly understood (Berti *et al*., 2014).

Fig. 6: Transfer of Glucose, Amino Acids and Fatty Acids across the Placenta



BM - basal membrane; **EL** - endothelial lipase; **FA** - fatty acid; **FABP** - fatty acid binding protein; **FABPpm** - plasma membrane fatty acid binding protein; **FAT/CD36** - fatty acid translocase; **FATP** - fatty acid transport protein; **FFA** - free fatty acid; **GLUT** - glucose transporter; **LAT** - large neutral amino acid transport; **LPL** - lipoprotein lipase; **MVM** - microvillous membrane; **TG** - triglycerides; **X** - exchangers

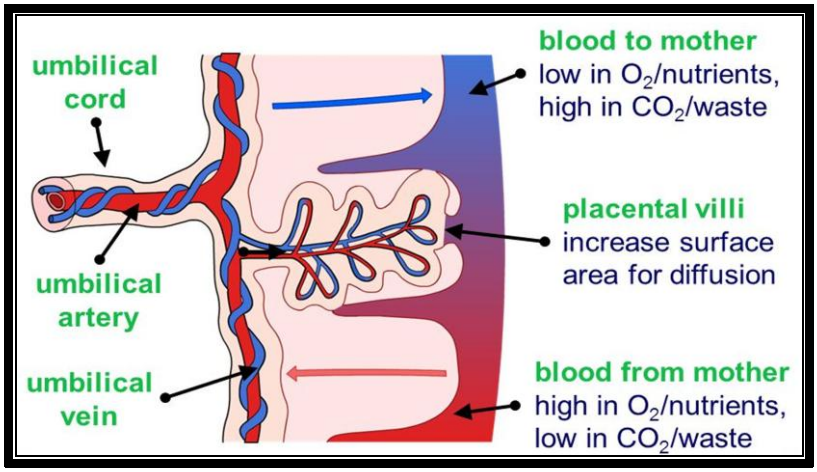
Source: Modified from Brett et al., Int. J. Mol. Sci., 2014; 15, 16153-16185

During the first trimester, foetal development takes place in a low oxygen environment and is supported by histiotrophic nutrition (in which local macromolecules are chiefly responsible for the maintenance of the embryo) from the endometrial glands (Burton *et al.*, 2002). Proper trophoblast invasion results in the opening of spiral and uterine glands towards the intervillous space enabling hemotrophic nutrition (which results from a transfer of material between the maternal and foetal circulations) in the second and third trimesters of pregnancy (Huppertz *et al.*, 2014). In addition to nutrition the foetus also depends on the mother for supply of oxygen.

1.2.3 Oxygen Transfer to the Foetus via Placenta

During pregnancy both the placenta and foetus require high amounts of oxygen (Mutinati *et al*., 2013). The placenta allows passage of oxygen from mother to foetus by passive diffusion (Mayhew, 2014) (Fig. 7). The placenta lacks neuronal innervation suggesting that local physical (e.g., oxygenation; flow rate), paracrine (e.g., endothelial cell nitric oxide) and circulating (e.g., angiotensin II) factors contribute to blood flow regulation in small foeto-placental vessels (Wareing *et al*., 2014). The placenta not only transfers oxygen to the foetal circulation but also consumes oxygen in order to support its own energy demands for the processes of nutrient transport, protein synthesis and growth (Mayhew, 2014). The reactive oxygen species (ROS) that are generated as a result of metabolism participate in cell survival, proliferation and apoptosis (Bevilacqua *et al*., 2012).

Fig. 7: Passive Diffusion in the Placenta



Source: © Boardworks Ltd. 2007, http://www.boardworks.co.uk/

In the first trimester the extravillous cytotrophoblast (CTB) cells occlude the uterine spiral arterioles creating a low oxygen environment early in pregnancy, which is essential for the trophoblast invasion and the embryo development (Schneider, 2011). Hypoxia is a key signal for normal placental development in pregnancy and has a crucial role in the control of trophoblast differentiation into invasive or

proliferating cells (Doridot *et al*., 2013). The low oxygen environment ensures the rapid growth of embryo. It protects the embryo from teratogenesis which is mediated by oxygen free radicals and also keeps the maternal circulating immune cells in blood away from the developing placenta (Huppertz *et al*., 2012). Towards the end of the first trimester, blood flow and oxygenation rise within the placenta, supporting an increased capacity for mitochondrial oxidative metabolism in both the placenta and developing foetus (Murray, 2012).

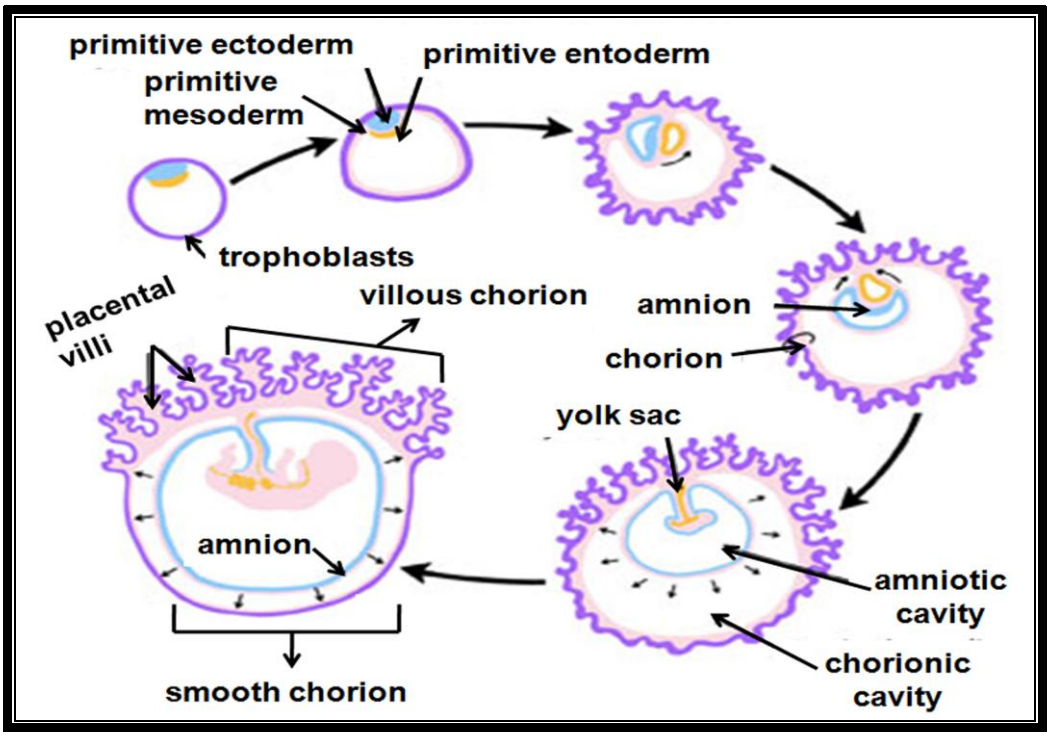
The proper development of placenta is essential as it plays an important role in embryonic and foetal growth and is discussed below.

1.3 Development of the Placenta

The formation of the embryo begins with the fertilized ovum which undergoes mitosis and gives rise to a multicellular structure called the blastocyst (Castro-Rendón *et al*., 2006). Embryonic stem cell (ESC) lineages in the inner cell mass, form the embryo and trophoblast stem cells in the trophectoderm of extra embryonic cells develop into the placenta (Bischof and Irminger-Finger, 2005) (Fig. 8).

The specification of the trophoblast lineage and the subsequent formation of the placenta are among the earliest differentiation events which take place in mammalian development (Rai and Cross, 2014). Trophoblast stem cells are differentiated into proliferating, polarized epithelial cell CTB and then onwards into villous CTB of anchoring villi and non-proliferating syncytiotrophoblasts (STB) (Logan *et al*., 2013). Subsequently the extravillous trophoblasts invade the maternal decidua and remodels spiral arteries reaching as far as the inner third of the myometrium (Pollheimer and Knöfler, 2012).

Fig. 8: Formation of Placenta and Foetal Membranes



Source: Pacific Research Centre for Early Human Development, Hawaii, USA. http://www2.jabsom.hawaii.edu/PRCEHD/placenta_development.html

The trophoblast invasion leads to increased uterine and umbilical blood flow required for vascularization of the placenta (Kliman *et al*., 2000). This process provides stability to the placenta and also results in efficient utero-placental blood flow (Sharp *et al*., 2010). After the trophoblast invasion and widening of the maternal spiral arterioles in the first trimester the maternal blood replaces plasma flowing through intervillous space of the placenta (Huppertz *et al.*, 2012). After the blood flow is established, placental villi are developed and matured by angiogenesis and vascularization in the placenta (Huppertz and Peeters, 2005).

Reports suggest that any perturbations in the processes of placental development lead to variations in the supply of nutrients and oxygen to the foetus and program key systems (Kadyrov *et al*., 2013). These programming effects influence the risk of diseases in later life (Barker and Thornburg, 2013).

1.3.1 Abnormal Placental Development

It is well established that many pregnancy related problems such as preeclampsia and preterm labour appear to have their origins early in pregnancy. Most pregnancy complications arise from abnormalities in implantation and placental development (Norwitz, 2007). Shallow trophoblast invasion and defective vascular remodelling of the uterine spiral arteries in the first trimester may result in impaired placental perfusion and chronic placental ischemia and hypoxia later in gestation leading to adverse pregnancy outcomes (Pringle *et al*., 2010). The premature loosening of the trophoblast plugs and consequent premature and disorganized flow of maternal blood into the intervillous space are known to be associated with recurrent miscarriage (Hempstock *et al*. 2003). Preeclampsia and intra uterine growth restriction (IUGR) are known to be associated with inadequate spiral artery remodelling (Pijnenborg *et al*. 1991).

1.4 Preeclampsia

Preeclampsia is defined as a pregnancy disorder characterized by high blood pressure (≥140/≥90 mm Hg) and an abnormal amount of protein in the urine (>1 + on a dipstick test) in a previously normotensive pregnant women (ACOG, 2014). The condition is associated with a reduced plasma volume, hemoconcentration and increased vascular resistance (Haram *et al*., 2014). The clinical findings of preeclampsia can manifest as a maternal syndrome with hypertension and proteinuria with or without other multisystem abnormalities or foetal syndrome that includes IUGR, reduced amniotic fluid and abnormal oxygenation (Sibai *et al*., 2005). If preeclampsia remains untreated, it moves towards a more serious condition known as eclampsia that includes systemic endothelial dysfunction, microangiopathy and

HELLP syndrome (haemolysis, elevated liver enzymes, and low platelet) (Al-Jameil *et al*., 2014). Early onset preeclampsia (≤34 weeks) is known to be associated with greater perinatal and maternal mortality and morbidity as compared to the late onset disease (≥34 weeks) (Soto *et al*., 2012).

The underlying mechanisms leading to preeclampsia remain elusive (Sones *et al.,* 2014) and the primary treatment for the disorder is delivery of the placenta. In view of this, there is a pressing need to better understand the mechanisms of the disease, with the ultimate goal of preventing the disorder (Ilekis *et al*., 2007) especially since the incidence of preeclampsia and rates of adverse outcomes are increasing (Gilbert *et al*., 2014).

1.4.1 Risk Factors for Preeclampsia

The risk factors of preeclampsia include maternal smoking, pre-existing medical conditions such as hypertension, diabetes mellitus, older maternal age and obesity (Hutcheon *et al*., 2011). Other risk factors of preeclampsia include nulliparity, first pregnancy after age of 35 years, thrombotic vascular disease, multiple gestations, proteinuria and a prior history of preeclampsia (Al-Jameil *et al*., 2014). Further ethnicity also is a risk factor as African-American women are reported to have a higher risk of mortality from hypertensive disorders of pregnancy compared with Hispanic, American Indian/Alaska Native, Asian/Pacific Islander, and Caucasian women (Lo *et al*., 2013).

1.4.2 Epidemiology of Preeclampsia

Preeclampsia is a leading cause of maternal and perinatal morbidity/mortality (Zuniga *et al*., 2014). It affects about 5-10% of all pregnancies, affecting a total of

about 8.5 million women worldwide (Telang *et al*., 2013; Soto *et al*., 2012). The prevalence of preeclampsia in developing countries ranges from 1.8% to 16.7% (Osungbade and Ige, 2011). WHO estimates the incidence of preeclampsia to be seven times higher in developing countries (2.8% of live births) than in developed countries (0.4%) (WHO, 2013). The HELLP syndrome occurs in 0.5% to 0.9% of all pregnancies and in 10% to 20% of women with severe preeclampsia (WHO, 2013; Haram *et al*., 2009).

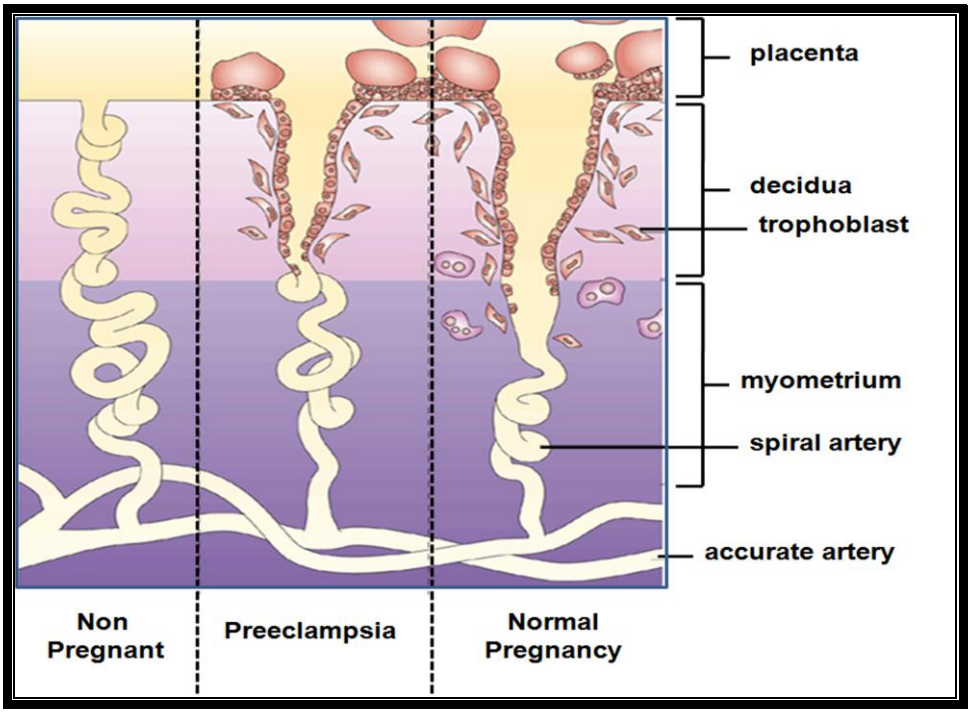
In addition to mortality, maternal morbidity is associated with preeclampsia (Kane *et al*., 2013). Women with preeclampsia are at an increased risk of later cardiovascular disease and neurological manifestations such as blindness, persistent neurological deficits secondary to stroke and later cognitive impairment (Brown *et al*., 2013; Aukes *et al*., 2007). Further, babies born to women with preeclampsia are at a twofold increased risk of neonatal mortality, or are born preterm and/or growth restricted and are susceptible to long term neurological disability as well as cardiovascular and metabolic diseases in later life (Davis *et al*., 2012; Basso *et al*., 2006; Barker *et al*., 2002). The neurological complications of preeclampsia and eclampsia are responsible for a major proportion of the morbidity and mortality both for women and their infants (Kane *et al*., 2014).

1.4.3 Pathophysiology of Preeclampsia

The two stage model of preeclampsia proposes that a poorly perfused placenta (stage 1) produces factor(s) leading to the clinical manifestations of preeclampsia (stage 2) (Redman *et al*., 2014). In preeclampsia the maternal spiral arteries fail to be invaded or remodelled by foetal cytotrophoblasts, resulting in constricted, high-resistance vessels altering the blood flow to the placenta (Powe *et al*., 2011).

Inadequate spiral artery remodelling leading to poor placental development and disturbed angiogenesis are currently regarded as the underlying pathophysiology of preeclampsia (Rosser and Katz, 2013; Moffett-King, 2002) (Fig. 9).

Fig. 9: Trophoblast Invasion



Source: Moffett-King, Nature Reviews Immunology, 2002; 2, 656-663

1.4.3.1 Placental Ischemia/ Hypoxia

Placental ischemia is a condition in which blood flow to the tissues is restricted while in hypoxia tissues are deprived of adequate oxygen supply. Placental ischemia induces the release of growth factor inhibitors, anti-angiogenic factors, inflammatory cytokines, ROS and hypoxia-inducible factors (HIF) which in turn generate widespread dysfunction of the maternal vascular endothelium (Reslan and Khalil, 2010). Chronic hypoxia during gestation may adversely affect the normal adaptation of uterine vascular tone and increase the risk of preeclampsia (Zhu *et al*., 2013). Hypoxia in the placenta is associated with vascular remodelling, hypertension, metabolic changes, oxidative stress, mitochondrial dysfunction and endoplasmic reticular stress (van Patot *et al*., 2012).

1.4.3.2 Altered Angiogenesis

Inadequate uterine angiogenesis/vascularity (decidualization) at the time of implantation is suggested to lead to preeclampsia (Torry *et al*., 2004). Vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and their antagonist's soluble fms-like tyrosine kinase-1 (sFlt-1, also known as sVEGFR1) and soluble endoglin (sEng) are involved in vasculogenesis of the placenta (Kar, 2014). VEGF exerts its biologic effects through two high-affinity tyrosine kinase receptors i.e. vascular endothelial growth factor receptor-1 (VEGFR-1) / fms-like tyrosine kinase-1 (Flt-1) and VEGFR-2 / kinase insert domain containing receptor (KDR) (Holash *et al.*, 2002).

Reports indicate that an imbalance between angiogenic and anti-angiogenic factors can cause preeclampsia (Savaj and Vaziri, 2012). Antiangiogenic factors secreted from abnormally perfused placenta are instrumental in mediating maternal endothelial dysfunction and consequent symptoms of preeclampsia (Cindrova-Davies, 2014). Increased serum sFlt-1 and decreased PlGF levels are known to be associated with a shorter duration of pregnancy, foetal growth restriction and preterm onset of the disease (Xia *et al*., 2003). Studies from our department have indicated that dysregulation of angiogenic factors in preeclampsia may be associated with maternal oxidative stress, reduced cord docosahexaenoic acid (DHA) levels and increased sFlt-1 levels, leading to poor birth outcomes (Kulkarni *et al*., 2010).

1.4.3.3 Immunological Intolerance and Inflammation

Insufficient trophoblast invasion and poor spiral artery remodelling in preeclampsia have been linked to immune dysregulation (Norris *et al*., 2011). Endothelial dysfunction observed in preeclampsia is suggested to be part of an

exaggerated systemic inflammatory response that involves maternal leukocytes and proinflammatory cytokines (Faas *et al*., 2014; Raghupathy, 2013). Various signalling molecules responsible for the propagation of an inflammatory response have been identified in placental cells which have to be explored in pregnancy complications (Weiss *et al*., 2009).

1.4.3.4 Genetics

Although most cases of preeclampsia occur in women without a family history, the presence of preeclampsia in a first-degree relative increases a woman's risk of severe preeclampsia two to fourfold (Carr *et al*., 2005). Two preeclampsia susceptibility genes such as activin receptor type 2 gene (ACVR2A) and storkhead box 1 (STOX1) gene have been identified within confirmed regions with significant genome-wide linkage (van Dijk and Oudejans, 2011). Catechol-O-methyltransferase (COMT) is an enzyme involved in catecholamine and estrogen degradation and is suggested to have a role in development of preeclampsia (Roten *et al*., 2011). Allelic variations in the fms-like tyrosine kinase 1 and VEGF C genes are associated with preeclampsia in black women and white women (Srinivas *et al*., 2010).

1.4.4 Maternal Endothelial Dysfunction

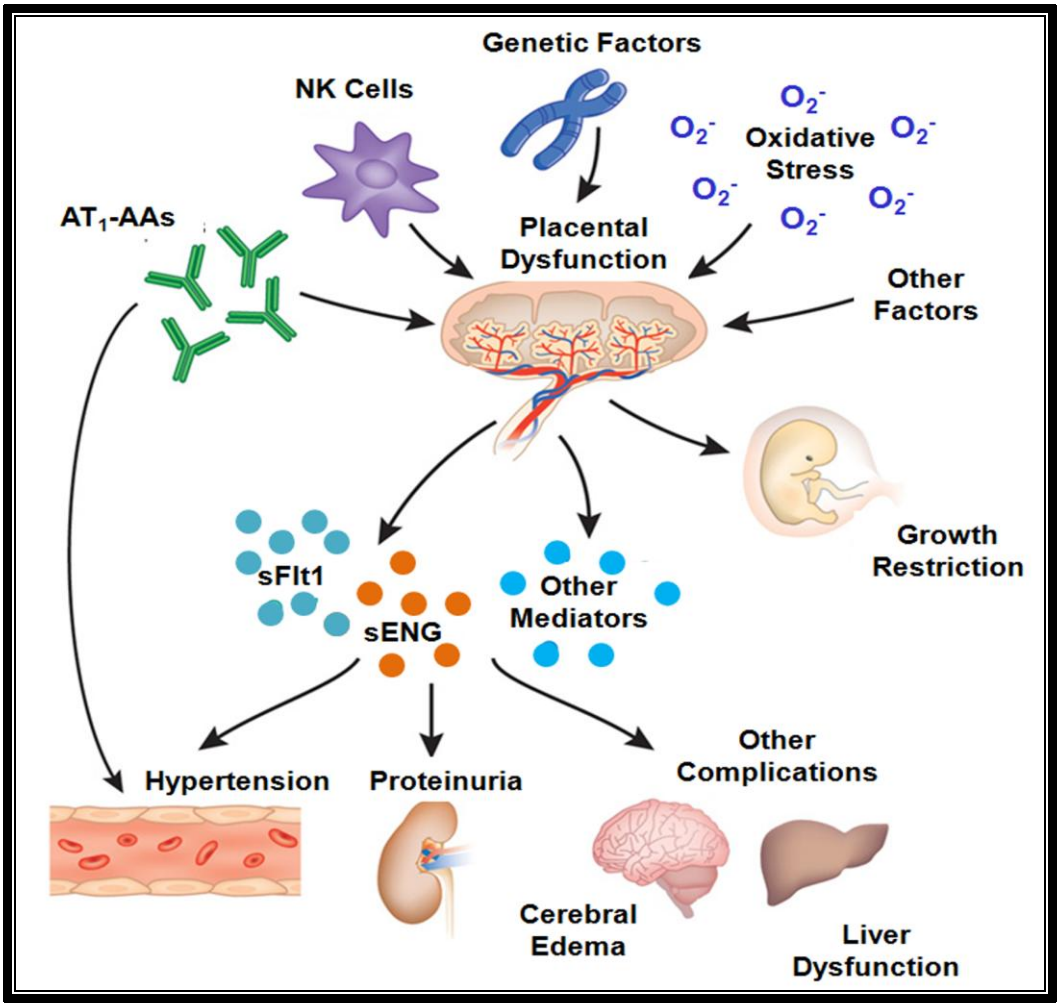
Oxidative stress on the endothelium leading to endothelial dysfunction is suggested to be the root cause of preeclampsia (Yelumalai *et al*., 2010). Reactive ROS are implicated in the pathogenesis of vascular dysfunction in preeclampsia (Steinert *et al*., 2006). It has been hypothesized that ROS or their metabolites ultimately compromise the vasodilatory, anti-aggregatory and barrier functioning of the vascular endothelium in preeclampsia (Hubel, 1998). Both the placenta and maternal

vasculatures are major sources of reactive oxygen and nitrogen species which can produce powerful pro-oxidants that covalently modify proteins and alter vascular function in preeclampsia (Lorquet *et al*., 2010).

During pregnancy, a critical balance exists between endothelium derived relaxing and contracting factors that maintain vascular homeostasis (Gilbert *et al*., 2008). When this delicate balance is disrupted, the vasculature is predisposed to vasoconstriction, leukocyte adherence, mitogenesis, prooxidation and vascular inflammation (LaMarca *et al*., 2008). A hypoxic placenta accelerates shedding of STB basement membrane fragments and other factors like leukocyte and platelet membrane particles, ROS, activated neutrophils, cytokines, growth factors, angiogenic factors and hormones into the maternal circulation causing endothelial dysfunction (Parikh and Karumanchi, 2008; Myatt, 2006).

Endothelial cell dysfunction is suggested to be the main cause of multiorgan failure (Brozović *et al*., 2012) (Fig. 10). Generalized damage to the endothelium of the maternal kidneys, liver and brain at the cellular level probably occurs following the release of vasopressive factors from the diseased placenta (Redman *et al*., 1991). Kidney function is particularly susceptible to the endothelial changes in preeclampsia that manifest functionally as vascular constriction, decline in renal blood flow, glomerular filtration rate and proteinuria (Moran *et al*., 2004). Cerebral edema and intracerebral parenchymal haemorrhage are commonly seen in women who die from eclampsia (Sibai, 2005). Liver and adrenals show infarction, necrosis and intraparenchymal hemorrhage in women with preeclampsia (Young *et al*., 2010). Further, endothelial dysfunction in preeclampsia has been attributed to placental oxidative stress (Redman and Sargent, 2005).

Fig. 10: Pathophysiology of Preeclampsia



AT1-AA's – angiotensin receptor agonistic autoantibodies; **NK cells** – natural killer cells; **O_2^-** - superoxide anion; **sENG** – soluble endoglin; **sFlt1** – soluble fms-like tyrosine kinase-1

Source: Parikh and Karumanchi, Nat Med, 2008 14:810-2.

1.5 Oxidative Stress

Stress that arises when the production of ROS overwhelms the intrinsic anti-oxidant defences is known as oxidative stress (Tadesse *et al*., 2014). Reactive species and free radicals are unstable molecules that oxidize other molecules in order to become stable (Gomes *et al*., 2012). It is known that although oxygen supports life it acts as a double edged sword (Greabu *et al*., 2008). Reactivity allows oxygen to participate in high-energy electron transfers during metabolism and hence supports the generation of large amounts of adenosine-5-triphosphate (ATP) through oxidative

phosphorylation (Burton and Jauniaux, 2011). Oxidative stress and oxidants play a critical role in defence against infection, tissue repair and signalling (Wahlqvist, 2013). However, oxygen can also react rapidly with most other radicals, forming ROS that cause selective oxidation of lipid, protein or DNA molecules (Loh *et al*., 2006; Lewén *et al*., 2000; Halliwell and Cross, 1994).

A continuing balance between oxidation and antioxidation is necessary for good health (Wahlqvist, 2013). Free radicals produced during metabolism are balanced by the body's endogenous antioxidant systems and by the ingestion of exogenous antioxidants (Rahman, 2007). The enzymatic detoxification mechanisms involve antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)], small molecular-weight antioxidants [Vitamin E, Vitamin C, glutathione (GSH), ubiquinone, beta-carotene] and adaptive mechanisms leading to antioxidant gene expression (Kalyanaraman, 2013). In the absence of antioxidants there is an accumulation of prooxidants which leads to oxidative stress (Poljsak, 2011).

1.5.1 Oxidative Stress in Pregnancy

Oxidative stress is required for the normal progression of embryonic, placental, foetal growth and development (Lappas *et al*., 2011). However, increased oxidative stress is implicated in the pathophysiology of many adverse pregnancy outcomes such as miscarriage, diabetes related congenital malformations, spontaneous abortions, preterm birth, preeclampsia, foetal growth restriction and low birth weight (Burton and Jauniaux, 2011; Poston *et al.*, 2011; Al-Gubory *et al.*, 2010). Oxidative stress induced placental dysfunction (Dennery, 2010), suppression of placental angiogenesis, endothelial damage, altered vascular function (Lorquet *et al.*, 2010;

Burton *et al.*, 2009), immune malfunction (Hannan *et al.*, 2011), myometrium damage (Khan *et al.*, 2010) are suggested to underlie pregnancy complications.

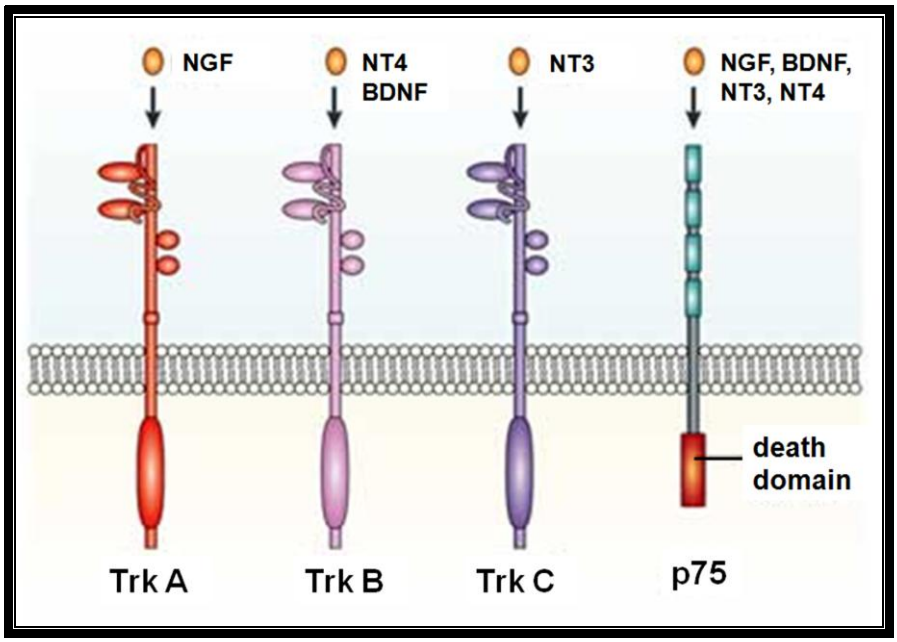
Increased oxidative stress is suggested to influence growth factors such as neurotrophins.

1.6 Neurotrophins

Neurotrophins are a family of growth factors that are required for the proliferation, differentiation, survival and death of neuronal cells in the nervous system (Li and Zhou, 2013). The four neurotrophins – nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin -3 (NT-3) and neurotrophin-4 (NT - 4) each bind and activate one or more of the tropomyosin-related receptor kinase (Trk) family of receptor tyrosine kinases (Trk A, Trk B and Trk C) (Reichardt, 2006; Chao, 2003) (Fig. 11). In addition, all neurotrophins activate the p75 neurotrophin receptor, a member of the tumour necrosis factor receptor family (Skaper, 2012). Through these receptors, neurotrophins activate many intracellular signalling pathways, thereby affecting both the development and function of the nervous system (Patapoutian and Reichardt, 2001). Expression of neuropeptides, small protein-like molecules (peptides) used by neurons to communicate with each other, depends on neurotrophins (Ernsberger, 2009; Petit *et al*., 2002; Nawa *et al*., 1993).

Apart from their role in the nervous system, studies have shown that neurotrophins promote angiogenesis, control the survival of adult endothelial cells, protect against oxidative stress and may have an important role in early vascular development in mother, placenta and foetus (Fujita *et al*., 2011; Caporali and Emanueli, 2009; Wu *et al*., 2004).

Fig. 11: Neurotrophins and their Receptors



BDNF - brain derived neurotrophic factor; **NGF -** nerve growth factor; **NT3 -** neurotrophin 3; **NT4 -** neurotrophin 4; **Trk -** tropomyosin-related receptor kinase

Source: Chao, 2003; Nat Rev Neurosci. 4:299-309.

Neurotrophins are naturally occurring molecules that regulate development of the placenta and brain. They play a crucial role in neurodevelopment (Shoval and Weizman, 2005). Further, neurotrophins can influence other growth factors and are involved in the vascular development and angiogenesis (Lazarovici *et al*., 2006). Growth factors such as neurotrophins have been demonstrated to act in a synergistic way in angiogenesis and neurogenesis contributing to self-healing powers of the adult human brain (Sopova *et al*., 2014). Variations in neurotrophins are suggested to have a role in the neurodevelopmental alterations and molecular mechanisms of cognitive dysfunction (Nieto *et al*., 2013). Differential patterns of cord blood neurotrophins have been observed in preterm pregnancies (Matoba *et al*., 2009)

The capacity of various neurotrophic factors to affect neurons is reported to be regulated during development (Knüsel *et al*., 1994). Studies suggest that neurotrophins form a link between maternal nutrient supply and foetal demand and

alterations in diet may lead to alterations in these growth factor levels through epigenesis (Dhobale and Joshi, 2012; Mayeur *et al*., 2011). The BDNF gene is reported to be under extensive epigenetic regulation (Schanker, 2012). Accumulating evidence suggests that epigenetic modifications of neurotrophins are associated with the pathophysiology of psychiatric disorders, such as schizophrenia and mood disorders (Ikegame *et al*., 2013). Reports indicate that oxidative stress and omega-3 fatty acids such as DHA affect the levels of neurotrophins (Bhatia *et al*., 2011; Kapczinski *et al*., 2008, Wu *et al*., 2008).

1.7 Maternal Nutrition, Oxidative Stress and Neurotrophins

Pregnancy is a state of increased requirement of macro- and micronutrients and malnourishment before and during pregnancy can lead to adverse perinatal outcomes (Imdad *et al*., 2011). Studies have adequately demonstrated the importance of maternal nutrition, particularly, micronutrients (folic acid, vitamin B_{12}) and long chain polyunsaturated fatty acids (LCPUFA) in determining pregnancy outcome (Khot *et al*., 2014). Various studies in our department have extensively discussed the link between folic acid, vitamin B_{12} and LCPUFA in the one carbon cycle (Dhobale and Joshi, 2012; Sundrani *et al*., 2011; Kulkarni *et al*., 2011)

1.7.1 Folate (Vitamin B_9)

Folates are a group of water-soluble B vitamins naturally existing in food such as legumes, green leafy vegetables and fruits (Mantovani *et al*., 2014). Folate in foods is naturally present in the form of reduced folate polyglutamate conjugates, while folic acid refers to the fully oxidized and most stable form of folate (Lucock, 2000). Folic acid is comprised of pteridine ring, p-aminobenzoic acid and glutamic acid

(Tamura and Picciano, 2006). Folic acid is required for DNA replication and as a substrate for a range of enzymatic reactions involved in amino acid synthesis and vitamin metabolism (Greenberg *et al*., 2011). Folate is critical for DNA, RNA and protein methylation as well as DNA synthesis and maintenance (Crider *et al*., 2012). Folate metabolism is known to affect ovarian function, implantation, embryogenesis and the entire process of pregnancy (Thaler, 2014). Folate deficiency is associated with hyperhomocysteinemia, megaloblastic anemia, embryonic and neural tube defects (NTD) (Guilland and Aimone-Gastin, 2013).

1.7.2 Vitamin B_{12}

Vitamin B_{12} is a crystallisable cobalt-complex, which belongs to a group of unique corrinoids, named cobalamins (Kräutler, 2012). The dietary sources of vitamin B_{12} are animal-source based foods, including meat, milk, eggs, fish, and shellfish (Watanabe, 2013). Vitamin B_{12} is a cofactor of methionine synthase in the synthesis of methionine, the precursor of the universal methyl donor S-Adenosylmethionine (SAM), (Gröber, 2013). It is required for the optimal functioning of the central and peripheral nervous system (Kumar, 2014). Low levels of vitamin B_{12} have been associated with neurocognitive disorders (Health Quality Ontario, 2013). Low maternal vitamin B_{12} status is known to be associated with increased risk of NTD, low lean mass, excess adiposity, increased insulin resistance and impaired neurodevelopment in the offspring (Rush *et al*., 2014).

1.7.3 Long Chain Polyunsaturated Fatty Acids

A fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. LCPUFA are the major components of brain and

retina and are the essential fatty acids with important physiologically active functions (Lee, 2013). There are two series of LCPUFA i.e. omega-3 and the omega-6 series. A complex series of desaturation and elongation reactions acting in concert transform the precursors linoleic acid (LA) of the omega-6 series and alpha linolenic acid (ALA) of the omega-3 series to their higher unsaturated derivatives: arachidonic acid (AA) and DHA respectively (Russo, 2009).

The demand for LCPUFA is increased during pregnancy because of the increasing needs of the foetus, mother and placenta (Makridis *et al*., 2011). Since their synthesis in the foetus and placenta is low, both the maternal LCPUFA status and placental function are critical for their supply to the foetus (Larqué *et al*., 2012). The LCPUFA, DHA is incorporated in large amounts in foetal brain and other tissues during the second half of pregnancy (Koletzko *et al*., 2007). Studies have claimed a beneficial effect of DHA supplementation on visual, neural or developmental outcomes (Campoy *et al*., 2012)

DHA, a major component of membrane phospholipids, plays an important role as an antioxidant agent (Suganuma *et al*., 2010). Recent reports indicate that omega-3 fatty acid supplementation reduced lipid peroxidation, nucleic acid and protein oxidation and promoted neuronal and glial cell survival in brain tissue of traumatic brain injury models (Kumar *et al*., 2014). Maternal dietary omega-3 fatty acid supplementation during pregnancy in rats is known to reduce placental oxidative damage and increase placental levels of pro-resolving mediators, which are associated with enhanced foetal and placental growth (Jones *et al*., 2014).

The ability of DHA to alter placental prooxidant/antioxidant balance is dependent on the DHA concentration used and the gestational age of the placental tissue (Stark *et al*., 2013).

1.7.4 Antioxidants

Supplementation with antioxidant micronutrients during pregnancy can be an early and innovative alternative to strengthen the prevention of chronic diseases in the population (Ramírez-Vélez *et al.*, 2011). Antioxidants including vitamin E, vitamin C, vitamin A, vitamin B_6, beta-carotene, zinc and selenium are known to have a beneficial role in reducing oxidative stress (Hsu and Guo, 2002) however their effect on oxidative stress markers has been inconsistent. Excessive vitamin A intake during gestation and lactation might be toxic for mothers with adverse effects for the developing offspring (Schnorr *et al*., 2011). Early observations suggest that vitamins C and E supplementation can prevent or ameliorate preeclampsia, but most large randomized clinical trials have failed to show any benefit (Talaulikar and Manyonda, 2011).

1.7.5 One Carbon Metabolism

B-vitamins like folate, vitamin B_{12} and vitamin B_6 are important components of the one carbon metabolism. Vitamin B_{12} and folic acid mediate the remethylation of homocysteine, which affects the production of the universal methyl donor, SAM (Herrmann and Obeid, 2007). S-adenosylmethionine (SAM) maintains methyl group supply for various macromolecules like DNA, neurotransmitters, proteins and membrane phospholipids (Umhau *et al.*, 2006). Inadequate enzyme activities and imbalances of substrates and cofactors in one carbon metabolism may cause homocysteine and S-adenosylhomocysteine (SAH) accumulation (Muskiet, 2005).

Hyperhomocysteinemia has been associated with oxidative stress (Kolling *et al*., 2011; Forges *et al*., 2007) and is proposed to play a role in the pathogenesis of preeclampsia, IUGR, preterm birth, spontaneous abortions and intrauterine foetal

death (Micle *et al*., 2012). The thiol group of homocysteine can auto-oxidize in circulation at physiological pH in the presence of oxygen leading to the production of hydrogen peroxide (H_2O_2), thereby generating oxidative stress (Jacobson, 2000).

Further, the synthesis of the cellular antioxidant, GSH is linked to the one carbon metabolic pathway. Homocysteine can indirectly result in oxidative stress by decreasing the transcription, translation and catalytic activity of GPx (Lubos *et al*., 2007). Any disruption in the one carbon cycle or chronic methyl group deficiency can also result in cell death as a result of an imbalance in the cellular antioxidant defence systems and increased oxidative stress (Bagnyukova *et al*., 2008).

Although a lot of importance has been given to the methyl donors, the methyl acceptors also play an important role in the one carbon metabolism. Membrane phospholipids are major methyl group acceptors and earlier reports from our department have elaborately discussed that reduced DHA levels may result in diversion of methyl groups towards DNA ultimately resulting in altered DNA and histone methylation which may lead to altered gene expression patterns (Dhobale and Joshi, 2012; Sundrani *et al*., 2011). Such alterations in gene expression during critical periods of development may result in complications during pregnancy and lead to adverse birth outcomes.

1.8 Genesis of the Thesis

As discussed in the above section changes in maternal micronutrients such as folate and vitamin B_{12} could lead to increased homocysteine, thereby increasing the oxidative stress and altering DNA methylation patterns. This may further lead to increased lipid peroxidation, altered expression of antioxidant enzymes and neurotrophins ultimately leading to endothelial dysfunction associated with

preeclampsia. Studies from our laboratory have shown changes in global DNA methylation in response to altered one carbon metabolism in animals (Kulkarni *et al*., 2011). Several other animal studies have also suggested that disturbances of the folate and methionine-homocysteine cycles have an impact upon the epigenetic regulation of gene expression and hence may program long-term physiology and metabolism of the offspring (Engeham *et al*., 2010; Kim *et al*., 2009). Moreover, it has been proposed that maternal factors such as endothelial function and oxidative stress are key mechanisms of both foetal metabolic alterations and subsequent development of non-transmissible chronic diseases (Ramírez-Vélez *et al*., 2011; Donkena *et al*., 2010).

Increased oxidative stress in pathologic pregnancies such as preeclampsia has important implications for placental function and foetal well-being (Lappas *et al*., 2011). This is of significance as adverse influences during foetal life are known to alter the structure and function of various cells, organ systems or homoeostatic pathways, resulting in increased risk for diseases in adult life (Jansson and Powell, 2007). Studies indicate that children born to mothers with preeclampsia are at increased risk for neurodevelopmental and metabolic disorders in later life (Herrera-Garcia and Contag, 2014; Silveira *et al*., 2007). Thus, there may be a link between maternal nutritional status, oxidative stress, foetal metabolic alterations and epigenetic programming of adult diseases in children born to mothers with preeclampsia.

A series of cross sectional studies carried out by our department earlier have demonstrated altered levels of folate and vitamin $B_{12,}$ reduced DHA levels, increased homocysteine and oxidative stress in pregnancy complications such as preeclampsia (Kulkarni *et al*., 2011; Mehendale *et al*., 2008). The department has further shown that DHA is negatively associated with homocysteine concentrations in preeclampsia (Kulkarni *et al*., 2011). Studies from our department also indicate increased oxidative

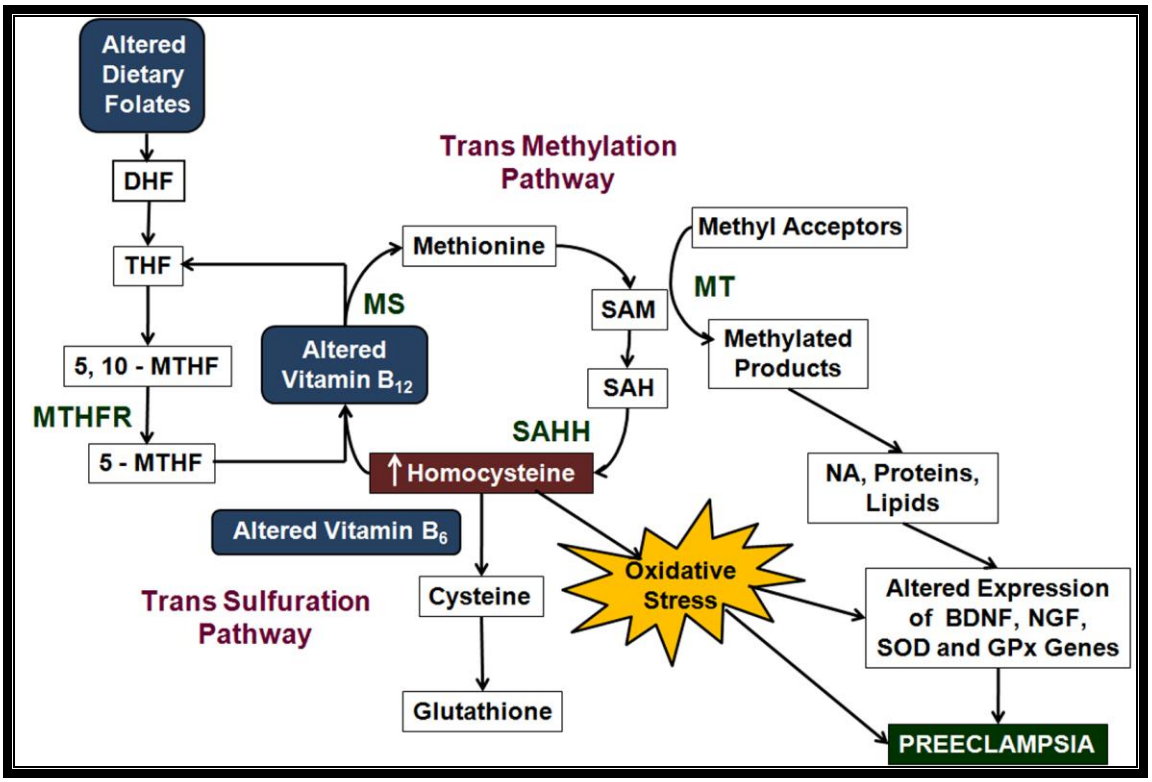
stress and lower levels of neurotrophins at the time of delivery in women with preeclampsia (Kilari *et al*., 2011; Mehendale *et al*., 2008). The increased oxidative stress has also been shown to be associated with altered angiogenesis leading to poor birth outcomes in preeclampsia (Kulkarni *et al*., 2010). It is however likely that these changes observed at delivery may be a result of secondary effects where the etiopathology has progressed for several weeks and months.

Further, it is known that the degree of vulnerability to changes in epigenetic patterns is high during early embryonic development (Gomes and Pelosi, 2013). The timing of environmental insults generating oxidative stress at critical periods of neurodevelopment may also play a decisive role in inducing neurodevelopmental disorders in the offspring (Do *et al*., 2009). A recent review indicates a need for prospective cohort studies to collect data on relevant clinical, environmental and lifestyle risk factors and longitudinal measurement of several factors such as angiogenic factors, neurotrophins and genetic variations in candidate gene pathways to establish interactions which predispose to pregnancy complications (Andraweera *et al*., 2012). Therefore, in order to better understand the role of oxidative stress and neurotrophins in preeclampsia it is important to prospectively examine their levels across gestation.

1.9 Hypothesis

With the above background we hypothesize that, **"Oxidative stress, through the dysregulation of the one carbon cycle leads to altered levels and expression of antioxidant enzymes and neurotrophins in preeclampsia" (Fig. 12)**

Fig. 12: One Carbon Metabolism and Oxidative Stress in Pregnancy



5-MTHF - 5-methylene tetrahydrofolate; **5,10-MTHF** - 5,10-methylene tetrahydrofolate; **BDNF** – brain derived neurotrophic factor; **DHF** - dihydrofolate; **GPx** – glutathione peroxidase; **MS** - methionine synthase; **MT** - methyl transferase; **MTHFR** - methylene tetrahydrofolate reductase; **NA** - nucleic acids; **NGF** – nerve growth factor; **SAH** - S-adenosyl homocysteine; **SAHH** - S-adenosyl homocysteine hydrolase; **SAM** - S-adenosyl methionine; **SOD** – superoxide dismutase; **THF** – tetrahydrofolate

In order to test the above hypothesis, the present prospective study was carried out in women with preeclampsia and normotensive pregnant women. This study will help in understanding the association of the oxidative stress and neurotrophins from early pregnancy in women who develop preeclampsia.

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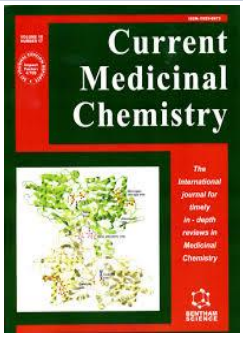

Counteracting oxidative stress in pregnancy through modulation of maternal micronutrients and omega-3 fatty acids

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Abstract

During pregnancy, oxidative stress has been implicated in the pathophysiology of preeclampsia and preterm birth leading to poor birth outcome. Hyperhomocysteinemia caused as a consequence of altered micronutrients like folic acid and vitamin B_{12} is associated with increased production of reactive oxygen species that generate oxidative stress. These micronutrients are important determinants of methyl donor, s-adenosyl methionine while phospholipids are important methyl acceptors in the one-carbon metabolic cycle. A series of our studies in women during pregnancy have demonstrated altered levels of these micronutrients and the negative association of docosahexaenoic acid with homocysteine. Various strategies to counteract oxidative stress during pregnancy such as antioxidant therapy have been examined and found to be inconsistent. In this review, we focus on the role of oxidative stress in pregnancy and discuss the possibility of ameliorating it through modulation of maternal micronutrients and omega 3 fatty acids especially docosahexaenoic acid. We propose for the first time that manipulation of one-carbon metabolism by maternal diet could be a potential mechanism to counteract oxidative stress through homocysteine lowering effects and help in reducing the risk for adverse pregnancy outcomes.

CHAPTER 2

Brain Derived Neurotrophic Factor Levels in Preeclampsia: A Cross Sectional Study

2.1 Introduction

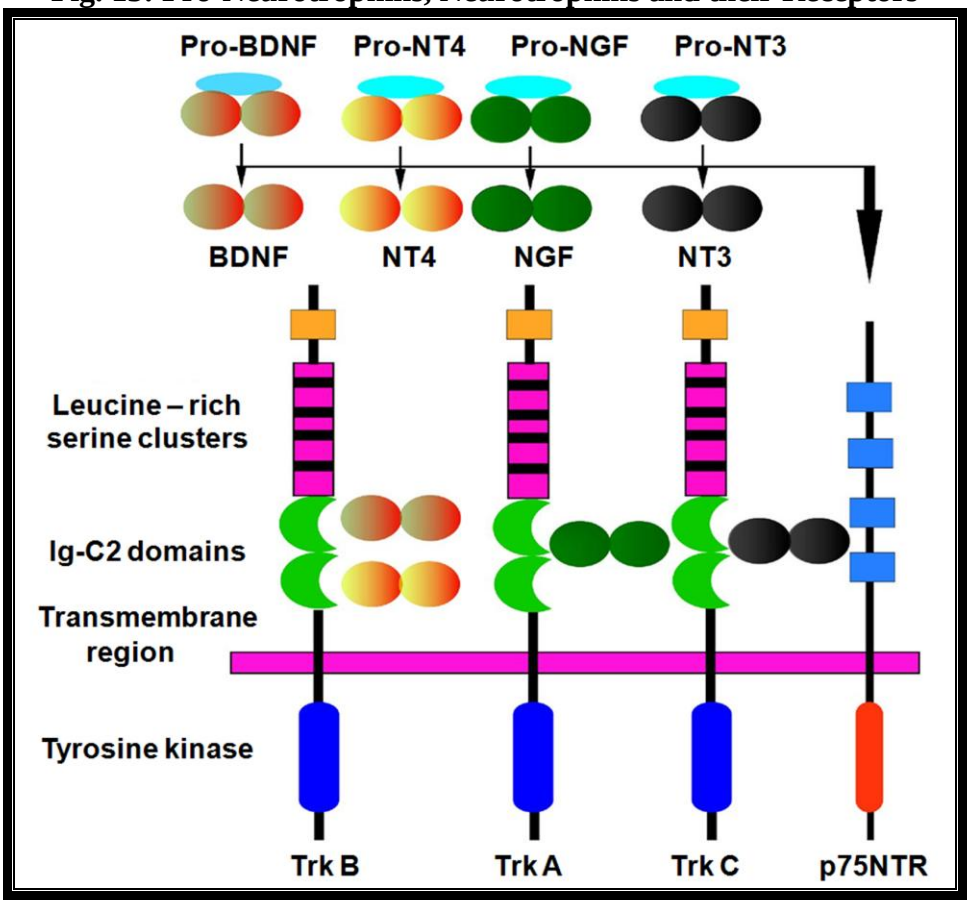
Neurotrophins are growth factors which regulate the development and function of the nervous system. Apart from their role in the nervous system neurotrophins are involved in initiation of vascular development, maintaining vessel stability and cardiovascular stability. Preeclampsia is a complex pathology that involves placental endothelial dysfunction. The brain derived neurotrophic factor (BDNF) is a neurotrophin implicated in pathologies related to placental and foetal growth disturbances and hence changes in its levels may contribute to the pathophysiology of preeclampsia. This chapter therefore examines the levels of BDNF at delivery in the mother and cord blood to better understand its role in preeclampsia.

2.1.1 Neurotrophins

The members of the neurotrophin family include nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) (Fargali *et al*., 2012). Each neurotrophin consists of a noncovalently-1 linked homodimer and contains (1) a signal peptide following the initiation codon; and (2) a pro-region containing an N-linked glycosylation site (Binder *et al*., 2004). Neurotrophins are translated from single coding exons and are synthesized as larger precursors (proneurotrophins) of ~30–34 kDa which are then proteolytically processed to generate small mature proteins (Teng *et al*., 2010). They exert their action by the activation of two distinct classes of transmembrane receptors; p75NTR and tropomyosin-related receptor kinase (Trk) family. Pro-forms preferentially activate p75NTR receptor that mediates apoptosis and the mature forms activate Trk family of receptors promoting survival of neuronal cells (Carvalho *et al*., 2008; Lee *et al*., 2001) (Fig. 13). Neurotrophins are

important regulators of neural survival, development, function and plasticity (Huang and Reichardt, 2001).

Fig. 13: Pro-Neurotrophins, Neurotrophins and their Receptors



BDNF - brain derived neurotrophic factor; **Ig** – immunoglobulin; **NGF** - nerve growth factor; **NT3** -neurotrophin 3; **NT4** - neurotrophin 4, **p75 -NTR** – p75 neurotrophin receptor; **Trk** - tropomyosin-receptor kinase

Source: Modified from Arévalo and Wu, 2006; Cell Mol Life Sci. 63:1523-37.

2.1.2 Neurotrophins in Pregnancy

Neurotrophins play important roles in influencing brain function from early age to adulthood (Molteni *et al*., 2001). By exerting neuroprotection, neurotrophins are critical for pre- and postnatal brain development (Malamitzi-Puchner *et al*., 2006). Apart from the nervous system, neurotrophins play numerous roles in angiogenesis, energy homoeostasis and regulation of other growth factors in the materno-feto-placental unit (Mayeur *et al*., 2011). Animal and cell culture studies have also

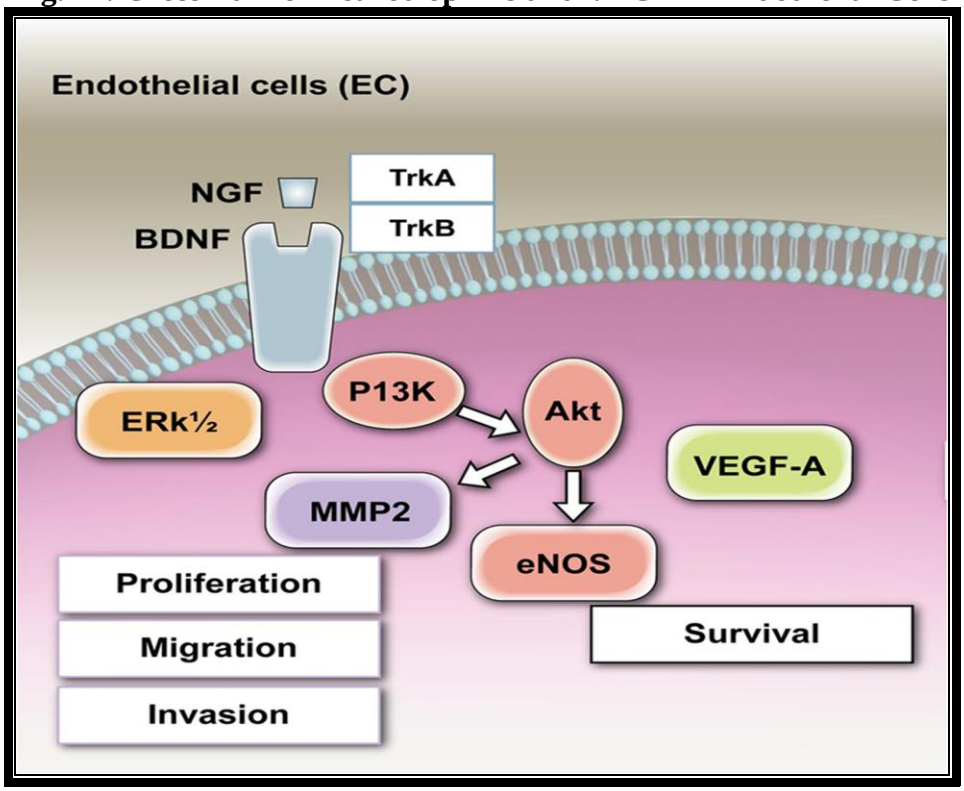
demonstrated that BDNF/Trk B signalling system plays a critical role in implantation, placental development and foetal growth (Kawamura *et al*., 2011). Reports indicate that the cytotrophoblast's differentiation, proliferation and survival of the placenta are regulated by neurotrophins (Mayeur *et al*., 2010; Kawamura *et al*., 2009; Kermani and Hempstead, 2007; Donovan *et al*., 2000), suggesting a role for BDNF in angiogenesis and placental development. Neurotrophins are important in the survival, maintenance and differentiation of neuronal tissue and functions in follicle maturation, tumour growth, angiogenesis and immunomodulation; however its role in the placenta remains unexplored (Fujita *et al*., 2011).

Studies from our department indicate dysregulation of neurotrophins like BDNF and NGF in normotensive preterm pregnancies (Dhobale *et al*., 2012a,b). Higher maternal BDNF and placental Trk B in women delivering preterm as compared to control indicated that BDNF protein may not be utilized for placental growth and development which leads to the increased levels in preterm deliveries. In another study from the department the NGF levels in women with preeclampsia were lower as compared to NC women indicating altered angiogenesis in these women (Kilari *et al*., 2011). Further, it is also reported that a reduction in cord BDNF and NGF levels may have implications for altered neurodevelopment in childhood and later life in children born preterm (Dhobale *et al*., 2012a, b). Abnormal neurotrophic factor gene activity is suggested to be a leading mechanism by which early life adverse experiences may persistently modify brain and behavioural plasticity (Roth and Sweatt, 2011).

2.1.3 Cross Talk between Growth Factors

Neurotrophins are suggested to be novel proangiogenic factors that can affect the endothelial cells as well as act through the myeloid progenitor cells (Duda and Jain, 2005). Literature indicates that there may be a crosstalk between neurons and endothelial cells via angiogenic factors, neurotrophins and their cognate receptors on both neurons and endothelial cells (Scharfman and MacLusky, 2008; Kim *et al*., 2004; Ogunshola *et al*., 2002) (Fig. 14).

Fig. 14: Cross Talk of Neurotrophins and VEGF in Endothelial Cells



BDNF – brain derived neurotrophic factor; **eNOS –** endothelial nitric oxide synthase; **ERk –** extracellular signal regulated kinase; **MMP –** matrix metalloproteinases; **NGF –** nerve growth factor; **PI3k - Akt –** phosphatidylinositol 3-kinase pathway; **Trk –** tropomysin - related receptor kinase; **VEGF –** vascular endothelial growth factor

Source: Modified from Caporali and Emanueli, 2009; Physiol Rev 89: 279-308.

BDNF can regulate development of peripheral organs such as angiogenesis in the heart and postnatal growth and repair of skeletal muscle (Varendi *et al*., 2014). It has been reported that BDNF contributes to neoangiogenesis by activating the Trk B

receptor expression in endothelial cells and by additionally recruiting bone marrow-derived cells (Kermani and Hempstead, 2007). BDNF is suggested to induce VEGF expression via HIF-1 alpha in neuroblastoma cells (Nakamura *et al*., 2006). BDNF has also been shown to play a role in the etiology of some cardiovascular diseases: induction of angiogenesis in ischemic tissues (Lorgis *et al*., 2009). Recent reports suggest that the two complementary processes, proangiogenic stimulation of new vessel growth and anti-angiogenic inhibition of vessel overgrowth, should be balanced to restore the vascular network in the placenta in any pregnancy complication (Chen and Zheng, 2014).

2.1.4 Factors Influencing Neurotrophins

There are various factors which influence neurotrophin levels and are discussed below

2.1.4.1 Nutrition

Undernutrition and overnutrition are implicated in many disorders of mental health and neurology and these actions are mediated by changes in energy metabolism and multiple signalling molecules such as neurotrophins (Dauncey, 2012). Nutrients like vitamins, minerals, polyunsaturated fatty acids (PUFA) and trace elements are needed for the normal metabolism of neurotrophic factors. However, the exact relationship among these factors and their interaction with genes and proteins concerned with brain development and growth is not well understood (Das, 2013). High saturated fat and high sugar has been shown to reduce BDNF levels in schizophrenia (Peet, 2004). Animal studies in our laboratory have shown that maternal diets imbalanced in micronutrients like folic acid and vitamin B_{12} reduced

brain DHA and BDNF levels (Sable *et al*., 2013). Reports from our department further indicate that a combination of omega 3 fatty acids and vitamin B_{12} enriched diet influence neurotrophin levels and improved cognition (Rathod *et al*., 2014).

Reports indicate that DHA and AA are essential nervous system components that increase in concentration throughout gestation and may influence neurotrophin mediated foetal brain development (Benn *et al*., 2014). LCPUFA upregulate the expression of neurotrophins and their target receptors (Balogun and Cheema, 2014) and have an influence on brain structure and function, including cognition (Bourre, 2006). Neuroprotectin D1 (NPD1), a bioactive anti-inflammatory and anti-apoptotic molecule formed from free DHA synthesis, is upregulated by neurotrophins and downregulated by oxidative stress (Bazan *et al*., 2010; Das, 2008).

Omega-3 fatty acids regulate neurotrophins that are important for the proper functioning of the central nervous system (CNS) (Wu *et al*., 2004) and are required for normal neurological development (Pandya and Yakel, 2013). Neurotrophins are very susceptible to the effects of dietary manipulations and diets rich in omega-3 fatty acids are known to upregulate BDNF while high energy diets do the opposite (Tyagi *et al*., 2013). Further, neurotrophins play a role in neuronal metabolism and synaptic plasticity and can be epigenetically regulated by diets rich in omega-3 fatty acids (Gomez-Pinilla and Tyagi, 2014).

2.1.4.2 Lifestyle factors

Stress-induced remodelling of the hippocampus, prefrontal cortex and amygdala in the brain is reported to be associated with changes in the levels of neurotrophins (Gray *et al*., 2013). Further stress-induced alterations in growth factors such as neurotrophins have been identified in modulating stress associated pathologies

(Bath *et al*., 2013; Nowacka and Obuchowicz, 2013). Studies suggest that neurotrophins plays a role in the development of stress vulnerability and resilience in the pathophysiology of major depression (Dwivedi, 2013; Masi and Brovedani, 2011).

Alcohol consumption during pregnancy is known to result in impairments of the CNS that result in cognitive deficits (Boehme *et al*., 2011). Inherited variation in the *BDNF* gene due to alcohol dependence may promote early developmental differences in neuronal proliferation in the brain (Hill *et al*., 2011). Dietary factors influence the basic mechanisms underlying ethanol-induced functional alterations and the related neuropathology in the brain (Jaatinen and Rintala, 2008).

BDNF signalling is well known to be involved in adaptation to stress and stress-related disorders (Pardon, 2010). Exposure to drugs of abuse such as cocaine is known to modulate epigenetic regulation of *BDNF* gene expression (McCarthy *et al*., 2012).

Prenatal exposure to maternal cigarette smoking is reported to affect brain development and behaviour in the adolescent offspring (Lotfipour *et al*., 2009). This may be due to significant reduction in *BDNF* mRNA and BDNF protein levels in the brain of offspring due to epigenetic changes induced by cigarette smoking (Yochum *et al*., 2014).

Exercise is a beneficial lifestyle factor with public health implications. It has a positive influence on mood, cognition and memory retrieval as it is known to increase neurotrophin levels (Meeusen, 2014; Vega *et al*., 2011). BDNF levels in humans are significantly elevated in response to exercise and the magnitude of increase is exercise intensity dependent (Ferris *et al*., 2007). Exercise during pregnancy is suggested to have long-lasting effects on the offspring health (Rosa *et al*., 2013).

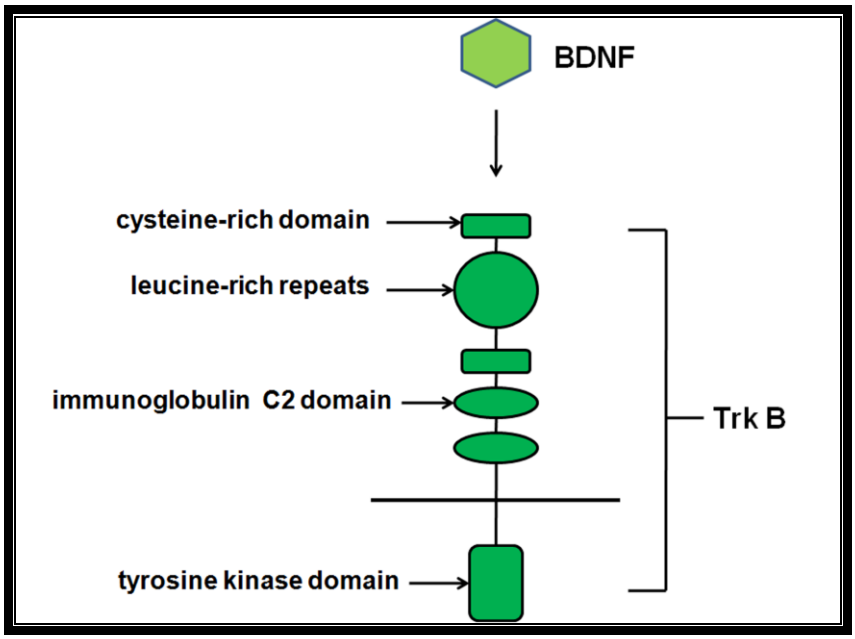
2.1.5 Brain Derived Neurotrophic Factor (BDNF)

BDNF, a secreted glycoprotein, shares about 50% amino acid identity with other neurotrophins. It is a noncovalently-1 linked homodimer having a signal peptide following the initiation codon and a pro-region containing an N-linked glycosylation site (Binder and Scharfman, 2004). It was first purified from the pig brain in 1982 (Barde *et al*., 1982). BDNF also shares a distinctive three dimensional structure containing two pairs of antiparallel β-strands and cysteine residues in a cystine knot motif with other growth factors (Binder and Scharfman, 2004).

BDNF is found in different areas of the CNS such as cortex, hypothalamus, hippocampus and amygdala (Andero and Ressler, 2012). It is also found in platelets, which bind, store and release BDNF upon activation (Fujimura *et al*., 2002).

It is well established that the synaptic effects of BDNF are attributed to Trk B activation (Lu and Nagappan, 2014) (Fig. 15). Activation of Trk B by BDNF mediates the survival and differentiation of neurons during development and is also required throughout adulthood to sustain the growth and function of neuronal synapses (Tessarollo, 1998). BDNF activation of Trk B is the key for the enhancement of activity-stimulated excitation of neurons that is needed for memory development and maintenance (Thiele *et al*., 2009).

Fig. 15: BDNF and its Trk B Receptor



BDNF – brain derived neurotrophic factor; **Trk B** – tropomyosin-related receptor kinase B

Source: Modified from Deinhardt and Jeanneteau, 2012 Protein Phosphorylation in Human Health 7: 217-232

2.1.6 BDNF Signalling Pathways

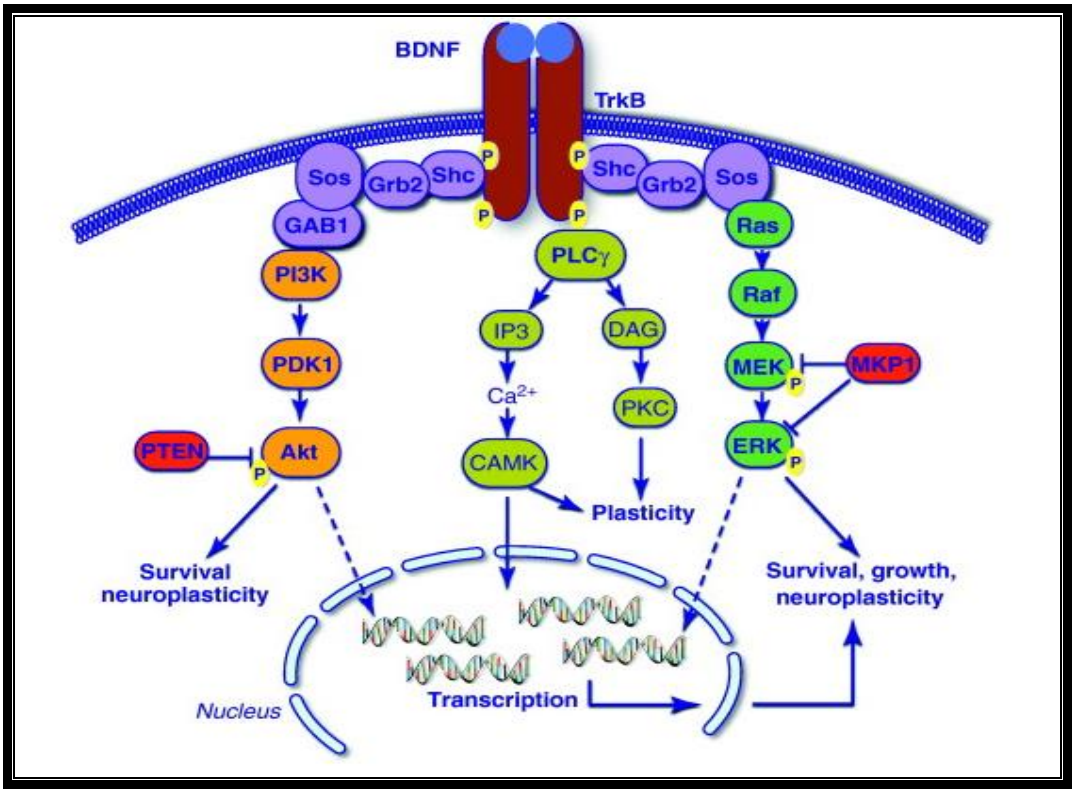
BDNF-induced dimerization of Trk B receptors leads to activation of the tyrosine kinase domain that initiates three major cascades of signalling pathways (Longo and Massa, 2013; Reichardt, 2006) (Fig. 16). They are as follows:

1) Mitogen-activated protein kinases (MAPKs),
2) Phosphoinositide 3-kinase (PI3K)
3) Phospholipase Cγ1 (PLCγ1)

Each of these signalling pathways also regulates several elements of gene transcription such as cAMP response-element binding protein (CREB) (Kim *et al*., 2013). CREB is a key component of diverse physiological processes, including nervous system development, cell survival, plasticity, as well as learning and memory (Lonze and Ginty, 2002). Several neurodegenerative disorders are a result of

dysregulation of the CREB transcriptional cascade possibly due to its effect on cell viability and cognitive function (Sakamoto *et al*., 2011).

Fig. 16: BDNF- Trk B Signalling Pathways



Akt - protein kinase B; **BDNF -** brain derived neurotrophic factor; **CAMK -** calmodulin-dependent protein kinase; **DAG -** diacylglycerol; **ERK -** extracellular signal regulated kinase; **GAB -** Grb2-associated binding protein 1; **IP3 -** inositol trisphosphate; **MEK –** mitogen activated protein kinase/ERK kinase; **MKP1 -** mitogen activated protein kinase phosphatase 1; **PDK1 -** 3-phosphoinositide-dependent protein kinase 1; **PI3K –** phosphotidylinositol 3-kinase; **PKC -** protein kinase C; **PLCγ –** phospholipase Cγ; **PTEN -** phosphatase and tensin homologue; **Ras, Raf -** rho-associated kinases; **Shc -** Src homology and collagen related molecules; **Sos -** son of sevenless proteins; **Trk B -** tropomyosin-related kinase B

Source: Duman and Voleti, 2012; Trends Neurosci. 35:47-56

2.1.7 Functions of BDNF in the Nervous System

BDNF is an important regulator of synaptogenesis and synaptic plasticity and is involved in mechanisms underlying learning and memory in the adult CNS (Cunha *et al*., 2010; Minichiello, 2009). BDNF is capable of mediating many activity-dependent processes in the mammalian brain, including neuronal differentiation and

growth, synapse formation and plasticity, and higher cognitive functions (Park and Poo, 2013). It is also known to regulate the fate of various cells axon growth, dendrite growth and pruning (Reichardt, 2006). It is known to support the survival of retinal ganglion cells and peripheral sensory neurons (Thoenen *et al*., 1987). Apart from these functions BDNF is involved in regulation of energy homeostasis, metabolic functions, eating behaviour and body weight (Rios, 2014; Noble *et al*., 2011; Rosas-Vargas *et al*., 2011).

2.1.8 BDNF and Neurodevelopment

BDNF influences cell proliferation, differentiation, survival and death of both neuronal and non-neuronal cells, thereby making it critical in the health and well being of the nervous system (Balaratnasingam and Janca, 2012). Developmental changes alter the neuronal responsiveness to certain neurotrophins, which subsequently are variously expressed, to properly balance their action and ensure protection to foetus (Ciccolini and Svendsen, 2001). BDNF is suggested to mediate the interactions between gene and environment, synaptic plasticity and apoptosis and influence transgenerational transmission of disease vulnerability (Balaratnasingam and Janca, 2012). Abnormal brain development in a compromised prenatal and/or early postnatal environment is thought to be a risk factor for several neurological disorders (Rehn and Rees, 2005). Changes in the levels of neurotrophins (growth factors) can produce long-lasting effects on neurotrophic processes (neuron number and synapse) that alter neuronal maturation and plasticity in later life (Vicario-Abejo´n *et al*., 2002). Reports indicate that low levels of BDNF are associated with mental disorders (Passaro *et al*., 2014; Duman and Monteggia, 2006).

Studies have shown that obstetric complications, particularly foetal hypoxia, leads to long-term alterations in brain structure and function that was in turn associated with increased risk of behavioural problems in children in later life (Cannon *et al*., 2008; Rehn *et al*., 2004). Manipulating the various pathways associated with BDNF may be a viable treatment approach to a variety of neurological and psychiatric disorders (Lu *et al*., 2014).

2.1.9 BDNF Levels in Preeclampsia

Preeclampsia is characterized by abnormal trophoblast invasion of the spiral arteries of the decidua and myometrium leading to a failure to establish an adequate uteroplacental blood flow giving rise to relatively hypoxic trophoblast tissue (Myatt, 2002). Recent reports indicate that abnormal development and dysfunction of the placenta due to changes in cell proliferation, differentiation and death induce preeclampsia (Gong and Kim, 2014). Growth factors like neurotrophins are critical in placental development (Malamitsi-Puchner *et al*., 2007; Quinn, 2005). BDNF and its receptor Trk B are expressed in rodent and human placenta supporting a role for BDNF in the regulation of several metabolic functions during pregnancy (Garcés *et al*., 2014). Alteration in the levels of BDNF during pregnancy may be associated with an abnormal development of the placenta resulting in preeclampsia. Children born to mothers with preeclampsia have consistently been suggested to be at risk for cognitive and behavioural disorders in later life (Kajantie and Räikkönen, 2010; Cannon *et al*., 2008). They also have a lower intelligence quotient and reduced cognitive performance at later ages (Ehrenstein *et al*., 2009; Many *et al*., 2003).

Further, preeclampsia is suggested to have two or more phenotypes of differing etiologies and manifestations (Phillips *et al*., 2010). The placentas of women

with preeclampsia onset before 34 weeks of gestation demonstrated placental findings predominantly consistent with insufficiency because of vascular abnormalities and were significantly different from those with preeclampsia at term (Nelson *et al*., 2014). Different subsets of preeclampsia having different pathophysiological mechanisms may contribute differently to the development of preterm versus term preeclampsia (Roberts and Catov, 2008). It is likely that the levels of neurotrophins in the cord may influence the neurodevelopment of the child. It is likely that BDNF is differentially regulated in term and preterm preeclampsia.

Therefore, the present chapter for the first time simultaneously compares the levels of BDNF both in maternal and in cord plasma samples in women with preeclampsia (delivering at term and preterm) and normotensive control (NC) women.

2.2 Materials and Methods

2.2.1 Participants

This study was conducted at the Department of Obstetrics and Gynaecology, Bharati Hospital, Pune and was approved by the Institutional Ethical Committee. A written informed consent was taken from each subject. A total number of 201 singleton pregnant women were recruited for this cross sectional study. This included NC women (n=95) and women with preeclampsia (n=106). Women with preeclampsia were further divided into term-preeclampsia (≥37 weeks gestation) (term-PE) (n=60) and preterm-preeclampsia (<37 weeks gestation) (preterm-PE) (n=46) groups.

Women were excluded from the study if there was evidence of other pregnancy complications, such as multiple gestation, chronic hypertension, type I or

type II diabetes mellitus, seizure disorder, renal or liver disease. Pregnant women with alcohol or drug abuse were also excluded from the study. The NC group consisted of pregnant women with no medical or obstetrical complications. Preeclampsia was diagnosed as per the ACOG standard criteria and was defined by systolic and diastolic blood pressure (BP) greater than 140/90 mmHg respectively, with the presence of proteinuria (>1+ or 300mg) on a dipstick test and recorded at 2 time points > 6 hour apart. Edema was present in some cases. This diagnosis of preeclampsia has been reported by our department in a series of earlier studies (Kulkarni *et al*., 2011; Kulkarni *et al*., 2010; Mehendale *et al*., 2008). Gestational age was based on day of last menstrual period and then confirmed by ultrasound. All women were from a lower socioeconomic group and had similar levels of education and lifestyle. Education was considered as the class/grade unto which the women had studied. None of the women smoked.

Mode of delivery was recorded for all the women. In the nomotensive control group, 70.7% deliveries were normal (spontaneous vaginal), 21.2% deliveries were by caesarean section and 5.1% were assisted deliveries. In the term-PE group, 58.2% deliveries were normal (spontaneous vaginal), 35.8% deliveries were by caesarean section and 6% were assisted deliveries. In the preterm-PE group 48.1% deliveries were normal, 50% deliveries were by caesarean section and 1.9% were assisted deliveries.

All biochemical measurements were carried out by the candidate and the intra assay coefficient of variability was determined by multiple aliquots of same sample and was less than 10%. The inter variability is routinely established with other investigators in the lab.

2.2.2 Sample Collection, Processing and Storage

Ten millilitres of maternal venous blood was collected at in ethylene diamine tetraacetic acid (EDTA) vials. In addition, 10 ml cord blood was also collected from the umbilical cord just after delivery. The blood was immediately layered on Histopaque (Sigma-Aldrich, St Louis, MO, USA) and centrifuged at 1800 rpm for 35 minutes to separate the plasma and erythrocytes. The erythrocyte fraction was washed 3 times with normal saline. Then, the plasma and erythrocyte aliquots were stored at -80°C until further analysis.

2.2.3 BDNF Assay

The BDNF assay was performed at a laboratory separate from subject recruitment sites. Investigators were blinded to subject identity which was indicated by a code number maintained by the clinical staff until analysis was completed.

BDNF levels were measured in both maternal and cord plasma using the BDNF Emax Immuno Assay System (Promega) and the method has been reported by our department earlier (Dhobale *et al.*, 2012a; Pillai *et al*., 2010). Briefly, the BDNF Emax Immunoassay system is designed for the sensitive and specific detection of BDNF in an antibody sandwich format. In this format, flat bottom 96-well plates are coated with anti-BDNF monoclonal antibody (mAb) which binds soluble BDNF. The captured BDNF is bound by a second, specific polyclonal antibody (pAb). After washing, the amount of specifically bound pAb is then detected using a species-specific anti-immunoglobulin Y antibody conjugated to horse radish peroxidase (HRP) as a tertiary reactant. The unbound conjugate is removed by washing and following incubation with a chromogenic substrate. The colour change is measured spectrophotometrically using enzyme-linked immunosorbent assay (ELISA) reader.

The amount of BDNF in the test solutions is proportional to the colour generated in the oxidation–reduction reaction. BDNF concentrations are expressed as pg/mL.

2.2.4 Power of the Study

There are no studies which have reported BDNF concentrations in preeclampsia. The statistical power was calculated using plasma BDNF values from our earlier study where we have reported significant group differences ($p<0.05$) in BDNF concentrations in plasma from 31 drug-naive first-episode psychotic subjects and 36 controls (Pillai *et al.*, 2010). This indicates that the difference in BDNF concentrations between patients and controls is indeed large. Thus, with such an effect size we proposed the sample size of 60 women with term-PE, 46 women with preterm-PE and 95 NC women which would give greater than 90 % probability of detecting a difference at an alpha of 0.05.

2.2.5 Statistical Analysis

Values are mean ± SD. The data was analyzed using SPSS/PC + statistical package (Version 20, Chicago IL). The data was checked for normal distribution by testing for skewness and kurtosis. Skewed variable i.e. cord BDNF was transformed to normality using the following transformations: log to the base 10. Mean values of the various parameters were compared using one way analysis of variance (ANOVA) and the post-hoc least significant difference (LSD) test. Contribution of covariates to differences in 3 populations was assessed by analysis of covariance. The actual difference between BDNF levels in preeclamptic and normotensive women after adjusting for gestational age was carried out using a linear regression model. Pooled

data from the whole cohort was used to test the association between maternal BDNF and blood pressure after adjusting for body mass index and gestation.

2.3 Results

2.3.1 Maternal and Neonatal Characteristics

Table 1 shows the maternal and neonatal characteristics. Both systolic and diastolic BP in the term and preterm-PE groups were significantly higher ($p<0.01$) than the NC group. Gestation and placental weights were reduced ($p<0.01$ for both) in the preterm-PE group as compared to the NC and term-PE groups. The neonatal characteristics like the baby weight, head circumference and chest circumference were significantly lower ($p<0.01$) in the term and preterm-PE groups as compared to the NC group.

Table 1- Maternal and Neonatal Characteristics

	NC (n=95)	Term-PE (n=60)	Preterm-PE (n=46)	PE (n=106)
Maternal Characteristics				
Age (years)	22.8 ± 3.4	23.3 ± 3.8	23.8 ± 4.5	23.3 ± 3.9
Income (INR)	5210 ± 2984	5402 ± 2674	5194 ± 2990	5280 ± 2835
Education (grade)	9.7 ± 3.4	10.2 ± 2.8	9.4 ± 3.6	9.8 ± 3.4
BMI (kg/m^2)	22.3 ± 3.0	22.0 ± 3.5	21.8 ± 4.2	21.9 ± 3.8
Systolic BP (mmHg)	123.4 ± 6.6	147.9 ± 12.5**	157.8 ± 17.6**##	151.9 ± 15.7**
Diastolic BP (mmHg)	78.4 ± 5.3	97.8 ± 9.4**	104.6 ± 13.7**##	100.8 ± 12.1**
Total gestation (weeks)	39.1 ± 1.1	38.77 ± 1.3	34.2 ± 2**##	36.75 ± 2.0**
Placental wt (gm)	513.4 ± 86.0	510.5 ± 96.0	422.5 ± 114.6**##	476.2 ± 128**
Neonatal Characteristics				
Baby Weight (kg)	2.9 ± 0.3	2.7 ± 0.4**	1.9 ± 0.5** ##	2.3 ± 0.6**
Baby Length (cm)	48.6 ± 2.4	47.9 ± 3.0	44.6 ± 3.9** ##	44.4 ± 4.0**
HC (cm)	34.2 ± 1.4	33.2 ± 1.8**	30.8 ± 3.0** ##	32.13 ± 2.8**
CC (cm)	32.6 ± 1.5	31.5 ± 2.1**	27.8 ± 2.9** ##	29.8 ± 3.2**

Values are expressed as mean ± S.D.; **p<0.01 (compared to NC); ## p<0.01 (compared to Term-PE), **BMI -** basic metabolic index; **BP -** blood pressure; **CC -** chest circumference; **HC -** head circumference; **NC -** normotensive control; **PE -** preeclampsia whole cohort; **Preterm-PE** - preterm preeclampsia; **Term-PE -** term preeclampsia

2.3.2 Maternal BDNF Levels

The mean maternal plasma BDNF levels were lower ($p<0.01$) in women with preeclampsia (term and preterm) than in the NC group. The data on control women (n=95) has been reported by us recently (Dhobale *et al*., 2012a). These differences in BDNF levels remained significant ($p<0.01$) even after adjusting for gestational age (Table 2). Further dividing the women with preeclampsia into term and preterm-PE and adjusting for gestation indicated that the mean maternal plasma BDNF levels were similar in the term and preterm-PE group.

2.3.3 Cord BDNF Levels

The mean cord plasma BDNF levels were higher ($p<0.05$) in the preeclampsia group (term and preterm) as compared to the NC group. After adjusting for gestational age these differences in BDNF levels remained higher ($p<0.05$) in preeclampsia as compared to the NC (Table 2). Further dividing the preeclampsia group indicated that cord plasma BDNF levels were higher ($p<0.01$) in the term-PE group as compared to the preterm-PE group. After adjusting for gestation, cord BDNF levels in the term-PE group continued to remain higher ($p<0.01$) as compared to the preterm-PE group.

Table 2- Maternal and Cord BDNF Levels

Maternal BDNF (pg/ml)	**NC (n=95)**	**Term-PE (n=60)**	**Preterm-PE (n=46)**	**PE (n=106)**
Without adjusting for gestation	458.5 ± 151.6	384.9 ± 97.7**	372.5 ± 86.8**	379.6 ± 92.9**
Adjusting for gestation	450.3 ± 152.3	379.6 ± 99.7**	396.6 ± 88.1*	386.9 ± 94.8**
Cord BDNF (pg/ml)	**NC (n=90)**	**Term-PE (n=54)**	**Preterm-PE (n=44)**	**PE (n=98)**
Without adjusting for gestation	609.2 ± 342.7	799.6 ± 391.3**	453.5 ± 126.8**##	644.2 ± 347.5*
Adjusting for gestation	573.4 ± 343.8	775.4 ± 396.3**	557.5 ± 136.3##	677.6 ± 325.5**

Values are expressed as mean ± S.D.; * $p<0.05$; **$p<0.01$ (compared to NC); ## $p<0.01$ (compared to Term-PE); **NC -** normotensive control; **PE** - preeclampsia whole cohort; **Preterm-PE** - preterm preeclampsia; **Term-PE -** term preeclampsia

2.3.4 Correlations between Maternal BDNF Levels and Maternal Blood Pressure

Maternal BDNF levels were negatively associated with the systolic blood pressure ($r = -0.176$, $p = 0.034$, $df = 143$) and diastolic blood pressure ($r = -0.261$, $p = 0.002$, $df = 143$) in the whole cohort.

2.4 Discussion

This study is novel, since we report for the first time both maternal and cord plasma BDNF levels in preeclampsia (both term and preterm) and compare them with NC women. Our results indicate (1) women with preeclampsia (term and preterm) had lower BDNF levels when compared to NC women. (2) Among the preeclampsia groups, maternal BDNF levels were similar in both term-PE and preterm-PE. (3) Cord

BDNF levels were higher in women with preeclampsia (term and preterm) when compared to NC group. (4) Among the preeclampsia groups, cord BDNF levels in the preterm-PE group were lower as compared to the term-PE group. The differences in BDNF levels remained significant even after adjusting for gestation.

2.4.1 Lower Maternal BDNF Levels in Preeclampsia

Our data indicates that mothers with preeclampsia had lower BDNF levels when compared to normotensive women. These findings are in contrast to earlier reports on a small sample size, which suggest higher maternal plasma BDNF levels in preeclampsia (Fujita *et al*., 2011). Other reports indicate that the maternal BDNF levels were similar in mothers delivering intrauterine growth retarded (IUGR) babies as compared to mothers delivering appropriate for gestational age babies (Malamitsi-Puchner *et al*., 2007). However, the above mentioned study included IUGR cases caused by preeclampsia, gestational hypertension, chronic diseases such as severe anemia, type I diabetes mellitus, hepatitis B, rheumatoid arthritis, renal insufficiency, asthma and psoriasis. Furthermore, the mothers in their cohort smoked cigarettes and it is well established that smoking affects BDNF levels (Kim *et al*., 2007). Since none of the women in our cohort smoked, our result mainly indicates the impact of preeclampsia on BDNF levels. Further, our study subjects were matched for age and dietary patterns all of which are known to confound the levels of neurotrophins (Lommatzsch *et al*., 2005).

We have earlier reported lower maternal VEGF levels in preeclampsia as compared to NC women (Kulkarni *et al*., 2010). It has been reported that there may be a cross-talk between the vascular and nervous systems (Li *et al*., 2006). We have recently reported higher maternal plasma BDNF levels (n=96, 505.26 ± 174.97 pg/ml,

$p<0.05$) and lower cord plasma BDNF levels in preterm deliveries (n= 96, 466.81 ± 227.63 pg/ml, $p<0.01$) as compared to term deliveries (Dhobale *et al*., 2012a). The levels of maternal BDNF in preterm-PE in the current study were lower as compared to those who delivered preterm without preeclampsia suggesting that the reduction of BDNF levels in maternal samples may be attributed to the altered placental pathology of preeclampsia. BDNF has been reported to play a role in embryo implantation, placental development and foetal growth in mice (Kawamura *et al*., 2009).

2.4.2 Differential Regulation of Cord BDNF in Term and Preterm Preeclampsia

The current data for the first time reports differential levels of cord BDNF in preeclampsia. Our findings highlight elevated cord BDNF levels despite reduced maternal BDNF levels in women with preeclampsia delivering at term. This may be due to either a preferential transport for brain-sparing in babies born to women with preeclampsia or impaired BDNF receptor signalling. Studies suggest that BDNF/Trk B signalling may play a protective role in the placenta whenever *in utero* conditions are unfavourable (Fujita *et al*., 2011).

The levels of cord BDNF in the current study were lower in women with preeclampsia delivering preterm. Similarly lower levels of cord BDNF has been reported by us earlier in women delivering preterm without preeclampsia. Infact, it has been suggested that there is a need to investigate the levels of BDNF in infants presenting various pathological conditions in the perinatal period (Nikolaou *et al*., 2006). One possible explanation for the findings in the current study may be that these women had lower placental weights which may lead to reduced placental flow. Chronic placental insufficiency has been shown to alter neurotrophin expression in the developing brain (Dieni and Rees, 2005).

Reduced BDNF levels observed in the current study may have implications for neonates born to women with preeclampsia delivering preterm since they may be at increased risk for neurodevelopmental disorders in later life. Earlier reports suggest similar levels of cord BDNF in both small for gestational age babies and appropriate for gestational age babies (Malamitsi-Puchner *et al*., 2006). SGA was caused by different clinical conditions which may confound the findings. It is also reported that lower BDNF concentrations in preterm neonates are indicative of prematurity (Malamitsi-Puchner *et al*., 2007). A study examined levels of neurotrophins in human umbilical cord blood from infants at different gestational ages and clinical conditions and suggested that cord plasma levels of BDNF may reflect the degree of neural maturity in premature infants (Chouthai *et al*., 2003). There is abundance of evidence in literature which shows that neurotrophic genes like BDNF and NGF may play a vital role in mediating processes linking early life environment and adult brain health (Roth and Sweatt, 2011; Branchi *et al*., 2006). In view of this, studies have been initiated in the department to follow-up infants born to women with preeclampsia for childhood or adult cognitive impairment.

Studies from our department have earlier reported increased oxidative stress and a consequential reduction in omega-3 fatty acids in preeclampsia (Mehendale *et al*., 2008). It is known that omega-3 fatty acids regulate levels of neurotrophins (Wu *et al*., 2004a). In addition, increased oxidative stress decreases serum BDNF concentrations (Kapczinski *et al*., 2008; Wu *et al*., 2004b). Oxidative stress increases BP which is suggested to increase sympathetic nerve activity (Gilbert *et al.*, 2008). A review by Hirooka *et al*. also highlights the role of oxidative stress in the pathogenesis of hypertension (Hirooka *et al.*, 2010). Increased oxidative stress and decreased BDNF levels are known to impair hippocampal neurogenesis in rats (Park *et al*.,

2010). The reduction in BDNF levels observed in the current study may be a consequence of increased oxidative stress and reduced omega-3 fatty acids in women with preeclampsia.

2.4.3 Negative Association of Maternal BDNF with Maternal Blood Pressure

In this study, maternal BDNF levels showed a negative association with blood pressure. BDNF has been suggested to work as a protective factor for endothelial cells in vasculature (Malamitsi-Puchner *et al*., 2006). Consequences of abnormal vascular development have been associated with preeclampsia which causes high BP (Malamitsi-Puchner *et al*., 2004). Preeclampsia involves endothelial dysfunction, platelet dysfunction/activation and sympathetic over-activity similar to cardiovascular disorders (Qiu *et al*., 2007). Epidemiological studies have demonstrated a relation between preeclampsia and an increased risk of maternal coronary heart disease and metabolic syndrome in later life (Craici *et al*., 2008; Ram and Santoro, 2005). BDNF has been shown to play a role in the etiology of some cardiovascular diseases and induction of angiogenesis in ischemic tissues. Reduced BDNF serum levels in patients with acute myocardial infarction or under cardiopulmonary bypass are related to oxidative stress and inflammatory response (Lorgis *et al*., 2010). Reduced maternal BDNF levels observed in this study may also be a predisposing factor for postpartum depression (Pinheiro *et al*., 2012) or cardiovascular disorders since reduced BDNF levels are also reported in metabolic syndrome patients (Chaldakov *et al*., 2004).

Our data highlights a role for BDNF in the pathology of preeclampsia and its differential effects on cord BDNF levels in women delivering preterm and term. The study suggests 1) reduced maternal BDNF levels in preeclampsia 2) differential regulation of cord plasma BDNF in women with preeclampsia delivering either term

or preterm. Based on our earlier reported data on angiogenesis reported earlier from our department and the reduced levels of BDNF in preeclampsia in the present study highlights the need to understand possible mechanisms underlying the association between angiogenesis and neurotrophins like BDNF in preeclampsia. Infants born to these mothers may present different pathologies in the neonatal period and it is important to follow up babies born to mothers with preeclampsia for cardiovascular risk or cognitive impairment in later life since they may be at risk for neurodevelopmental disorders.

This chapter examined the levels of BDNF at delivery in women with preeclampsia when the etiopathology has progressed for several weeks and months. Therefore, the next chapter examines the changes in BDNF levels in a prospective study at various time points across gestation both in normotensive women as well as in women who develop preeclampsia.

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Differential regulation of brain-derived neurotrophic factor in term and preterm preeclampsia

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Abstract

Our earlier studies in preeclampsia (PE) suggest a causal relationship between altered angiogenic factors and birth outcomes. Recent studies suggest that brain-derived neurotrophic factor (BDNF) can stimulate angiogenesis. The present study examines the levels of maternal and cord BDNF in women with PE (n = 106; full term [n = 60] and preterm [n = 46]) and normotensive women (n = 95; control) delivering at term. MaternalBDNF levels were lower (P < .05) in women with PE when compared to normotensive women. Cord BDNF levels were higher (P < .01) in women with PE delivering at term, while it was lower (P < .01) in women delivering preterm. Maternal BDNF levels were negatively associated with systolic and diastolic blood pressure (P < .01 for both). Our data for the first time suggest a possible role for BDNF in the pathophysiology of PE. Differential regulation of cord BDNF levels in preterm PE suggests a need to follow-up children to assess the neurodevelopmental effects in later life.

CHAPTER 3

Gestation Dependent Changes in Brain Derived Neurotrophic Factor Levels in Preeclampsia

3.1 Introduction

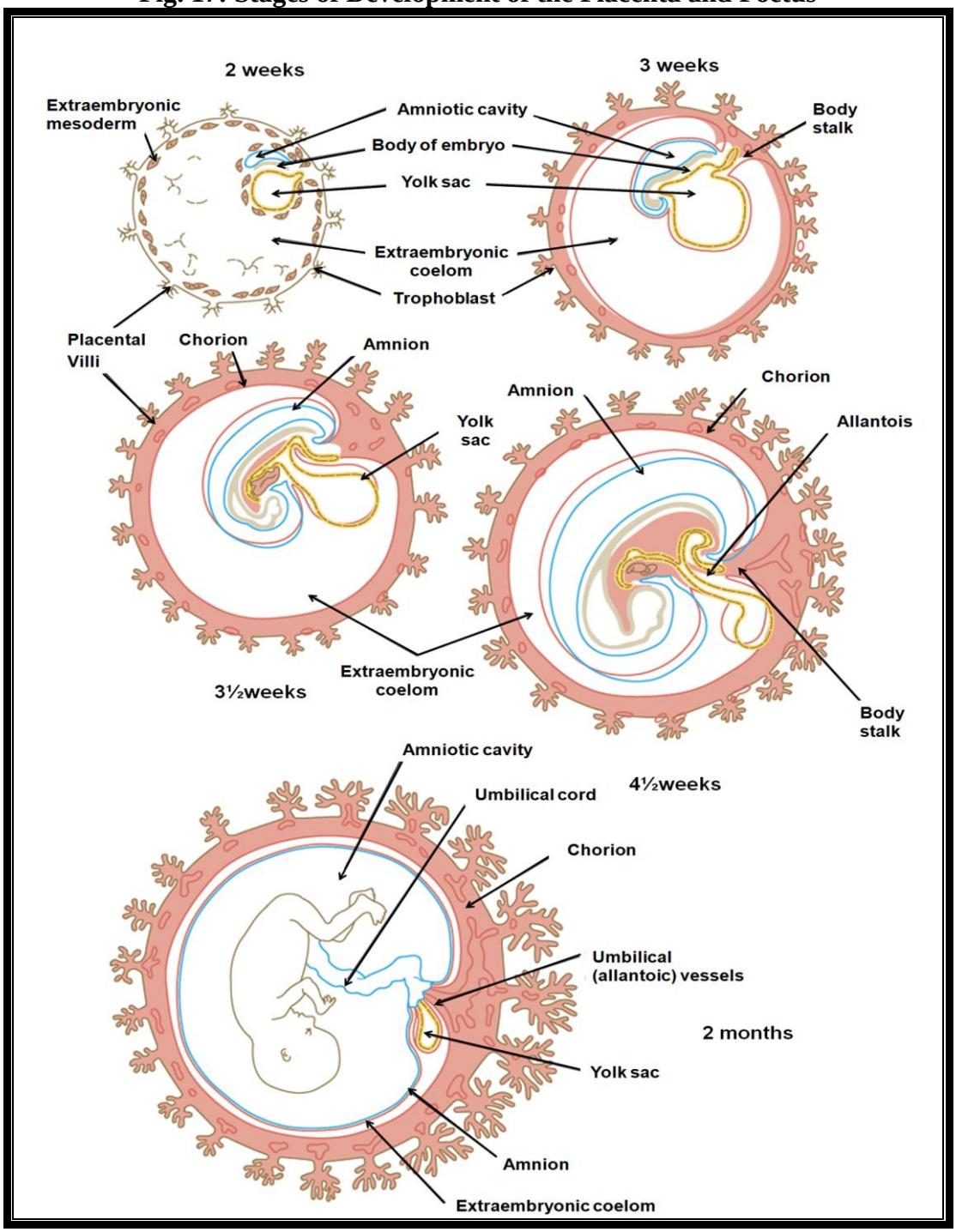
The last chapter compared the levels of BDNF in maternal and cord plasma samples of women with preeclampsia (delivering at term and preterm) and normotensive control women at the end of pregnancy. Studies indicate that neurotrophins play a critical role in implantation, placental development and foetal growth from early pregnancy. This chapter is a prospective study which examines the levels of maternal plasma BDNF from early pregnancy both in normotensive women and women with preeclampsia.

3.1.1 Placental Development across Gestation

In order to understand the role of BDNF in early placentation this section describes the development of the placenta across gestation. Normal placental development and function are essential for a successful pregnancy (John and Hemberger, 2012). Several steps result in the formation of the placenta: (1) The blastocyst attaches to the uterine epithelium and differentiates into an inner cell mass, an outer sphere of cells and the trophoblast (2) The trophoblast invades uterine tissues and spiral arterioles (3) Vascularization and branching angiogenesis in the placenta (Mess and Carter, 2007; Gude *et al*., 2004).

Early in mammalian development, the origins of trophoblast and embryonic cell lineages are established as the trophectoderm and the inner cell mass (ICM) in the blastocyst (Oda *et al*., 2006). The inner cell mass gives rise to the foetus and an outer layer of trophectoderm (TE) is the precursor of the placenta (Kimber, 2000) (Fig. 17).

Fig. 17: Stages of Development of the Placenta and Foetus



Source: Modified from Carlson BM: Patten's Foundations of Embryology, Ed 6, New York, 1996, McGraw-Hill, pg. 180.

The main functional units of the placenta are the chorionic villi within which foetal blood is separated by only three or four cell layers (placental membrane) from maternal blood in the surrounding intervillous space (Gude *et al*., 2004). Trophoblasts

that form the placenta provide the epithelial cover of the villous trees of the placenta and are in direct contact with maternal blood (Huppertz and Herrler, 2005).

During early pregnancy the placenta-derived trophoblasts start to invade the maternal uterus in order to regulate adequate blood flow and nutrient supply to the growing foetus (Pollheimer and Knöfler, 2012). The early invasive syncytiotrophoblast (STB) invades into uterine tissues (Zhang *et al*., 2013). Extravillous trophoblasts (EVT) first invade into the uterine decidua and then reach the myometrium (Lash *et al*., 2006). Subsets of the EVT invade into uterine spiral arteries (endovascular trophoblast) or uterine glands (endoglandular trophoblast) (Huppertz *et al*., 2014). Initially only maternal plasma flows through the intervillous space of the placenta in the first trimester which is replaced by maternal blood after the proper invasion of spiral arterioles (Huppertz *et al*., 2012).

Transplacental exchange, that depends on rates of uterine (maternal placental) and umbilical (foetal placental) blood flow, provides for all the metabolic demands of foetal growth and development (Reynolds and Redmer, 2001). This is mediated by two key processes, vascularization and angiogenesis, in the developing placenta (Huppertz and Peeters, 2005). Vasculogenesis is the process of formation of the earliest primitive capillaries and is achieved by differentiation of hemangiogenic stem cells that are derived from pluripotent mesenchymal cells (Burton *et al*., 2009). The subsequent process, angiogenesis, is characterized by the development of new vessels from already existing vessels and is a well coordinated process initiated by stimulation of various growth factors (Demir *et al*., 2010). Additionally, the developing placenta undergoes a process of vascular mimicry (referred to as pseudovasculogenesis) as cytotrophoblasts convert from an epithelial to an endothelial phenotype (Cerdeira and Karumanchi, 2012).

Placental development is well regulated by various autocrine/paracrine factors at the maternal-foetal interface (Chen, 2014). Every endothelial cell destined for vessel formation is equipped with receptors for several angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), hypoxia inducible factor-1 (HIF-1) and transforming growth factor (TGF) that regulates the process of neoangiogenesis (Gacche and Meshram, 2013). Patterns of placental angiogenesis are suggested to vary in pregnancy pathologies (Mayhew *et al*., 2004).

3.1.2 BDNF Levels in Pregnancy

It is well known that the neurotrophins receptors are mainly expressed in the nervous system. However, their mRNAs have also been detected in several utero foeto-placental tissues (Mayeur *et al*., 2011). BDNF has been shown to increase in the uterus before implantation and the expression of TrkB in trophectoderm cells of blastocysts suggests a potential paracrine role of BDNF in the blastocyst during implantation (Kawamura *et al*., 2007). After implantation, BDNF suppresses apoptosis in the embryo and promotes early embryonic development (Kawamura *et al*., 2007). It has also been reported that BDNF levels significantly decrease in the maternal serum before and after giving birth (Lommatzsch *et al*., 2006).

Altered BDNF levels have been observed in pregnancy complications. BDNF levels have been shown to be significantly elevated in the umbilical cord and is reported to be associated with increasing gestational age, reflecting the degree of neural maturity in premature infants (Chouthai *et al*., 2003). Furthermore, studies from our department have shown that the ratio of maternal/foetal BDNF levels are altered in preterm deliveries with the maternal levels being higher compared to levels in the umbilical cord (Dhobale *et al*., 2012a). Further, placental levels of the Trk B

receptor were significantly higher in the case of preterm deliveries (Dhobale *et al*., 2012a).

3.1.3 Longitudinal Changes of BDNF in Pregnancy

A recent longitudinal study in normotensive pregnant women demonstrates that serum BDNF levels in the first trimester are lower than those in the second and third trimester (Garcés *et al*., 2013). Alterations in BDNF levels may contribute to the early abnormal placentation in women with preeclampsia. However there are no longitudinal studies that have examined the levels of BDNF in women who develop preeclampsia.

As discussed in the earlier chapter, the cross sectional study demonstrated altered plasma BDNF levels at delivery in women with preeclampsia as compared to normotensive control (NC) women. It is likely that the changes at delivery may be secondary effects where the etiopathology has progressed for several weeks and months. Therefore, in order to better understand the role of BDNF in the pathology of preeclampsia, it is important to examine its levels in early gestation.

This study for the first time examines maternal BDNF levels across gestation, cord BDNF levels and placental *BDNF* gene expression in the complex pathology of preeclampsia. Their associations with the blood pressure (BP) of the mother and pregnancy outcome are also examined.

3.2 Materials and Methods

3.2.1 Participants

Pregnant women were enrolled for this longitudinal study from 2 major hospitals, Bharati hospital and Gupte hospital, in Pune, India. The research protocols and consent forms were approved by the Bharati Vidyapeeth Medical College Institutional Ethical Committee. Written consent was obtained from all the study subjects. This study is part of a large ongoing departmental study which recruits all consecutive pregnant women at 16-20 weeks of gestation who are willing to participate and give a written consent. The incidence of preeclampsia in this population is about 8-10% of total pregnancies. The first blood sample was taken between 16 - 20 weeks of gestation (T1), the second between 26 - 30 weeks of gestation (T2) and the third sample was taken at delivery (T3). Umbilical cord blood sample was also collected just after delivery. All women were followed up till delivery and categorized as preeclampsia if there was a presence of high BP (systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg) and proteinuria (>1+ or 300mg) on a dipstick test. BP was measured with a mercury sphygmomanometer, and preeclampsia was confirmed by repeated recording of the BP with an interval of 6 h at the time of diagnosis.

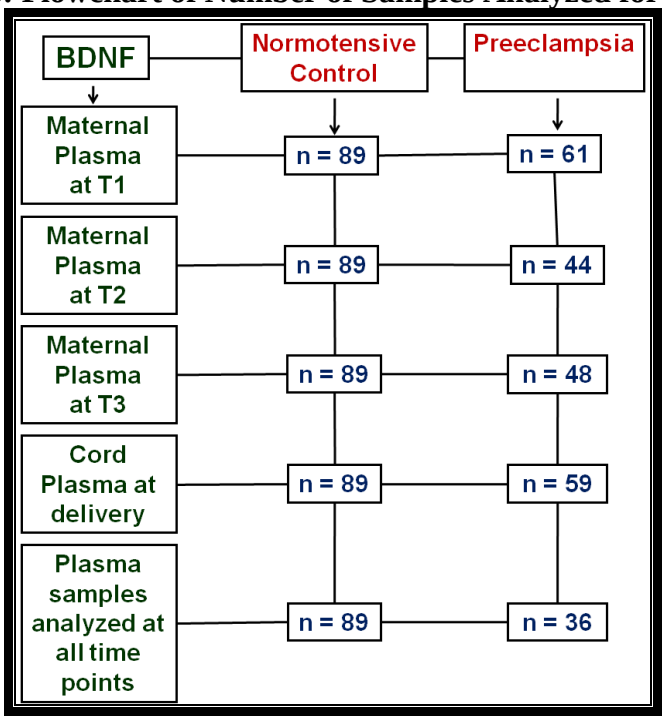
The pregnancies were dated by ultrasound as well as last menstrual period (LMP). The cases in which there were discrepancies of more than a week in gestational age between LMP and ultrasound were excluded from the study. Neonatal growth measures like birth weight, length, head circumference and chest circumference were recorded. Birth weight was recorded using a digital weighing scale (Zeal medical private limited, India) with an accuracy of 10 g. The length was measured to the nearest 0.1 cm using a portable Infantometer. The head circumference was measured using a measuring fiber glass measuring tape which was placed around

the head, just above the eyebrows anteriorly, and around the most prominent bulge posteriorly. The chest circumference was measured using fiber glass measuring tape which was placed around the lower chest. These procedures have been described in our earlier departmental study (Sundrani *et al*., 2013).

3.2.2 Power of the Study

Till date there are no studies examining plasma BDNF levels at various time points across gestation. This is the first longitudinal study of BDNF in women with preeclampsia. Therefore, we calculated the power of the study based on our earlier cross sectional study on BDNF levels in preeclampsia. Based on results from this study the Power and Sample Size Calculation version 3.0.43 software was used to calculate sample size of the current study. In the earlier chapter BDNF levels in NC (458.5 ± 151.6 pg/ml) and preeclampsia (379.6 ± 92.9 pg/ml) group were normally distributed having a population standard deviation = 122.25. If the true difference in the means of BDNF levels between preeclampsia and NC is 78.9, we would need to study 39 preeclampsia subjects and 78 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) 0.9. 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 89 NC and 61 preeclampsia women. (Fig. 18) Out of the 61 women with preeclampsia, 9 were severe, 2 showed IUGR and 10 delivered preterm.

Fig. 18: Flowchart of Number of Samples Analyzed for BDNF



BDNF – brain derived neurotrophic factor; **n** – number; **T1** - 16-20 weeks of gestation; **T2** - 26-30 weeks of gestation; **T3 -** at delivery

3.2.3 Sample Collection, Processing and Storage

10 ml of maternal venous blood was collected into the EDTA vials at all the time points while 10 ml of cord blood was collected at the time of delivery and was processed as described by us earlier in Chapter 2 Section 2.2.2.

Placental samples were collected on a random sub-sample. Thus, 21 preeclampsia and 22 control placental samples were collected and analyzed in this study. Fresh placental tissues were obtained on a sub sample from normal (n=22) and preeclamptic pregnancies (n=21) immediately after delivery. Briefly, the foetal membranes were trimmed off and small pieces were randomly cut out from the placental cotyledons. Tissue were rinsed in phosphate-buffered saline to wash off maternal and foetal blood, snap frozen in liquid nitrogen and stored at −80°C until assayed.

3.2.4 BDNF Assay

BDNF levels were measured in both maternal and cord plasma using the BDNF Emax Immuno Assay System (Promega). The details of this assay have been explained in Chapter 2, Section 2.2.3.

3.2.5 Extraction of Total RNA, cDNA Synthesis and Quantitative Real-Time (RT)-PCR Assays

Extraction of total RNA, cDNA synthesis and mRNA expression of *BDNF* and *GAPDH* genes from 22 NC and 21 preeclampsia placental samples was performed. The detailed method has been described by our department earlier (Meher *et al*., 2013). Total RNA from placenta samples was isolated using Trizol method and quantified by the Eppendorf Biophotometer plus (Hamburg, Germany). For each placental sample, 2 μg of RNA was reverse transcribed to cDNA using the High-Capacity cDNA reverse transcription kit (Cat No. 4368814, Applied Biosystems).

RT-qPCR for *BDNF* and *GAPDH* were performed with the TaqMan Universal PCR Master Mix (Cat No. 4324018, Applied Biosystems, California, USA) using the Applied Biosystems 7500 Standard Real Time PCR system. The relative expression level of the gene of interest was examined with respect to *GAPDH* to normalize for variation in the quality of mRNA and the amount of input cDNA. The real-time quantitative PCR reactions for each gene were performed in duplicate. To analyze the real-time quantitative PCR results, the average cycle number (Ct) of the reaction when it crossed a threshold value was determined for each reaction. Differences in Ct (ΔCt) between *GAPDH* and targeted gene *BDNF* were calculated by subtracting the Ct of the targeted gene *BDNF* from Ct of *GAPDH*. Relative expression levels of genes were calculated and expressed as $2^{\Delta Ct}$. The following TaqMan® Gene

Expression Assays (Applied Biosystems) were used in this study: *GAPDH* (Hs99999905_m1); *BDNF* (Hs00542425_s1).

3.2.6 Statistical Analysis

Continuous variables are reported as mean ± S.D. and discrete variables as percent (%). The data were checked for normal distribution by testing for skewness and kurtosis and BDNF values were found normally distributed. Differences in maternal and neonatal characteristics between preeclampsia and normotensive control (NC) were assessed using Student's t test for continuous variables and chi-square tests for categorical data. Pearson's correlation was used to examine the associations between BDNF levels and maternal age, body mass index (BMI), gestational age, parity, socioeconomic status (SES), education and income as they have been shown to affect BDNF levels (Pillai *et al*., 2012; Sher and La Bode, 2011; Rao *et al*., 2009; Zdanys *et al*., 2009; Macbeth *et al*., 2008; Lasky-Su *et al*., 2007; Lommatzsch *et al*., 2005). BDNF was correlated with maternal age, BMI and SES and hence BDNF levels were adjusted for these variables using multiple linear regression.

Associations of BDNF with BP and neonatal growth measures were analyzed after adjusting for maternal age, BMI and SES. The variable sample number (n) in different measures was either due to loss of follow up at various time points across gestation or insufficient sample volume available. Results corresponding to p-values lower than 0.05 are described as significant. The data were analyzed using the SPSS/PC+ package (Version 20.0, Chicago, IL, USA).

3.3 Results

3.3.1 Maternal and Neonatal Characteristics

The maternal body mass index (BMI) at all time points in women with preeclampsia (T1, $p<0.01$; T2, $p<0.01$; T3, $p<0.01$) was higher than NC women. Maternal systolic (T1, $p<0.01$; T2, $p<0.01$; T3, $p<0.01$) and diastolic BP (T2, $p<0.01$; T3, $p<0.01$) of women with preeclampsia was higher than NC women. The percentage of nulliparous women was higher in the preeclampsia (n=61) group compared to the NC (n=89) group (72% vs. 46%, $p<0.01$). There was no difference in education between groups (Table 3). Neonatal characteristics show that birth weight, head circumference and chest circumference was lower ($p<0.05$) in preeclampsia than in the NC group (Table 4).

Table 3: Maternal Characteristics

	NC (n=89)	**PE (n=61)**
Age in years	27 ± 4	27 ± 5
BMI in kg/m^2		
T1	22 ± 4	25 ± 5**
T2	24 ± 4	28 ± 6**
T3	26 ± 4	30 ± 6**
Gestation in weeks (range)		
T1	19 ± 2 (16 – 20)	18 ± 2 (16 – 20)**
T2	29 ± 2 (26 – 30)	29 ± 3 (26 – 30)
T3	39 ± 1 (37 – 41)	38 ± 2 (28 – 40)**
Sys BP in mmHg		
T1	111 ± 9	117 ± 12**
T2	112 ± 8	122 ± 13**
T3	120 ± 9	142 ± 18**
Dias BP in mmHg		
T1	72 ± 7	74 ± 8
T2	71 ± 7	76 ± 9**
T3	77 ± 6	94 ± 12**
Education in n (%)		
lower	28 (32)	13 (21)
higher	11 (12)	9 (15)
graduation	31 (35)	26 (43)
post graduation	19 (21)	13 (21)
Parity in n (%)		
nulliparous	41 (46)	44 (72)
multiparous	48 (54)	17 (28)
Income in INR (range)	3000 - 75000	5000-75000

Values are expressed as mean ± S.D.; **p<0.01 (compared to NC); **BMI** - body mass index; **Dias BP** - diastolic blood pressure; **NC** - normotensive control; **n –** number; **PE** - preeclampsia; **Sys BP** - systolic blood pressure; **T1** - 16-20 weeks of gestation; **T2** - 26-30 weeks of gestation; **T3** - at delivery

Table 4: Neonatal Characteristics at Birth

	NC (n=89)	PE (n=61)
Weight (kg)	2.9 ± 0.3	2.8 ± 0.4*
Length (cm)	48 ± 2.9	47.5 ± 2.3
Head Circumference (cm)	33.9 ± 1.3	33.2 ± 1.3*
Chest Circumference (cm)	32.2 ± 1.6	31.5 ± 2.5*

Values are expressed as mean ± S.D.; *p<0.05 (compared to NC); **NC -** normotensive control; **PE –** preeclampsia

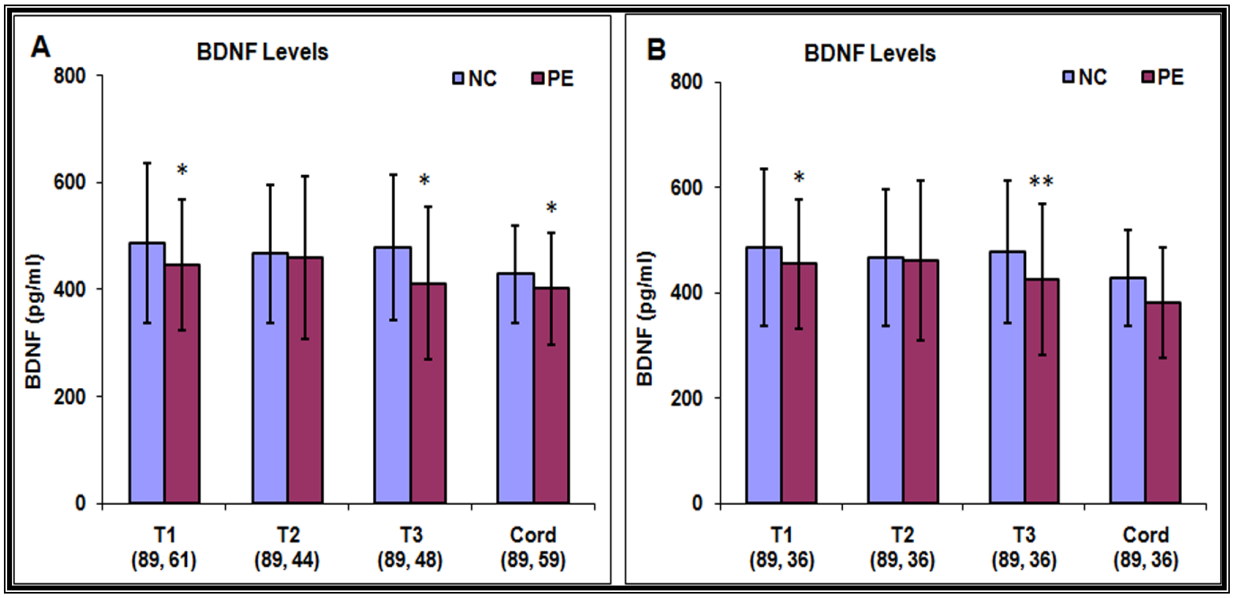
3.3.2 Maternal BDNF Levels

Maternal BDNF levels at T1 were lower (p<0.05) in women with preeclampsia (455 ± 128 pg/mL) as compared to NC women (486 ± 148 pg/mL). Maternal BDNF levels at T2 were comparable in women with preeclampsia (462 ± 151 pg/mL) and NC women (467 ±117 pg/mL). Maternal BDNF levels at T3 were lower (p<0.05) in women with preeclampsia (426 ± 118 pg/mL) as compared to NC women (478 ± 135 pg/mL) (Fig. 19).

3.3.3 Cord BDNF Levels

Cord BDNF levels were lower in preeclampsia (381 ± 76 pg/mL) (p<0.05) as compared to the NC group (429 ± 92 pg/mL) (Fig. 19).

Fig. 19: Maternal and Cord BDNF Levels across Gestation in Women with Preeclampsia as Compared to Normotensive Control Women

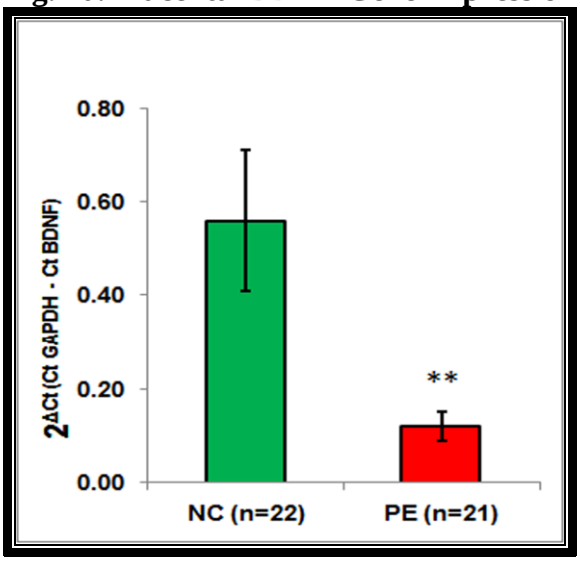


Values are expressed as Mean ± S.D.; *p<0.05, **p<0.01 as compared to NC. **A -** all samples; **B -** all timepoints; **BDNF -** brain derived neurotrophic factor; **NC -** normotensive control; **PE -** preeclampsia; **T1 -** 16 to 20 weeks of gestation; **T2 -** 26 to 30 weeks of gestation; **T3 -** at delivery

3.3.4 Placental *BDNF* Gene Expression

Placental *BDNF* gene expression was lower (p<0.01) in women with preeclampsia than in NC women (Fig. 20).

Fig. 20: Placental *BDNF* Gene Expression



Values are expressed as Mean ± S.E.; **p<0.01 as compared to NC; **NC -** Normotensive control; **PE** - Preeclampsia

3.3.5 Association between Maternal BDNF Levels and Maternal Blood Pressure at T3

Maternal systolic BP was negatively associated with BDNF levels at T3 (r = -304, p = 0.0050, n=82) in NC women. However, there was no association between maternal BP and BDNF in women with preeclampsia.

3.3.6 Association between Maternal BDNF Levels and Neonatal Growth Measures

There was no association between maternal BDNF levels at any time point and neonatal growth measures in both NC women and women with preeclampsia.

3.3 Discussion

This study for the first time reports levels of maternal plasma BDNF across gestation, cord plasma BDNF and placental *BDNF* gene expression in women with preeclampsia compared with NC women. Our results indicate that 1) Maternal BDNF levels were lower at T1 and T3 in women with preeclampsia than those in NC women 2) Cord BDNF levels were lower in the preeclampsia group as compared to NC group 3) Placental *BDNF* gene expression levels were also lower in women with preeclampsia than in the NC women.

3.4.1 Lower Maternal BDNF Levels at T1 and T3

This study reports lower maternal BDNF levels in women with preeclampsia at T1 and T3 as compared to NC women. As mentioned in chapter 2, we observed lower maternal BDNF and cord BDNF levels in preterm preeclampsia as compared to NC group at delivery in a cross sectional study. BDNF levels are known to be influenced by number of factors such as urbanicity, age, food intake and SES (Bus *et al*., 2011). Lower BDNF levels observed in this study may be a result of increased

oxidative stress and reduced omega-3 fatty acids like docosahexaenoic acid (DHA) both of which are known to influence the levels of BDNF (Bhatia *et al*., 2011; Kapczinski *et al*., 2008; Wu *et al*., 2008). Further, the studies in our department have reported increased oxidative stress at delivery in women with preeclampsia as compared with NC women (Mehendale *et al*., 2008). Similarly these studies have also demonstrated a negative association between DHA and BDNF in preterm pregnancy (Dhobale *et al*., 2012a) and lower levels of DHA in preeclampsia as compared to control in a cross sectional cohort (Kulkarni *et al*., 2011).

This data is a part of a large multi-investigator study where detailed information on nutritional intakes has been recorded and is reported separately where the frequency of consumption of omega 3 fatty acid (ALA and DHA) rich foods was similar in both groups at all time points (Wadhwani *et al*., 2014). Studies have shown that omega-3 fatty acids influence levels of neurotrophins (Sharma *et al*., 2012; Bhatia *et al*., 2011; Wu *et al*., 2004). Our earlier cross sectional studies indicate that there is no difference in the intake of omega-3 fatty acids between NC women and women with preeclampsia (Kulkarni *et al*., 2011). In addition, studies have shown that serum BDNF concentrations are season dependent (Molendijk *et al*., 2012; Comasco *et al*., 2011). However, in contrast others do not report seasonal changes in BDNF levels (Katoh-Semba *et al*., 2007). Three seasons define the Indian subtropical climate, summer (from February to May), the monsoon (from June to September) and winter (from October to January) and climatic variations in Pune, Western India are not drastic. In our study women from both groups have been recruited all around the year and the BDNF values at each time point are an average of women recruited across all the seasons. In view of this, it is unlikely that seasonality would affect the levels of BDNF. Further, it has been reported that in the tropical climate of western India,

seasonal variation is not associated with incidence of preeclampsia (Subramaniam, 2007).

It is known that the implantation of the blastocyst and early development of the placenta are crucial for a successful pregnancy (James *et al*., 2012) and neurotrophic factors like BDNF potentiate placental development (Kawamura *et al*., 2009). Recent reports suggest that as pregnancy progresses, the placenta and foetus may have a role in the production of BDNF (Garcés *et al*., 2013). Maternal BDNF is reported to reach the foetal brain through the utero-placental barrier and might contribute to its development (Kodomari *et al*., 2009). BDNF is known to stimulate angiogenesis in the endothelial cells in the placenta (Caporali and Emanueli, 2009). Further, reports indicate that during embryonic development BDNF expresses its TrkB receptor resulting in a direct angiogenic action on endothelial cells involved in cardiac vessel wall stability (Kermani *et al*., 2007). This may be due to a cross talk between neurons and endothelial cells via VEGF, neurotrophins and their cognate receptors on both neurons and endothelial cells (Li *et al*., 2006). Our findings suggest that reduced BDNF levels in early pregnancy may be partly responsible for reported abnormal foeto-placental development in preeclampsia.

3.4.2 Lower Placental *BDNF* Gene Expression and Cord BDNF Levels

In the current study, placental *BDNF* gene expression in women with preeclampsia was lower as compared to NC women. Studies indicate that *BDNF* is expressed in the human placenta, immunolocalized in the membranous chorion, trophoblast layer and endothelium, supporting its role in the regulation of several metabolic functions during pregnancy (Garcés *et al*., 2013; Fujita *et al*., 2011). It has been reported that the BDNF/Trk B signaling system is localized in the utero-foetal tissue in the placenta, suggesting a transient action of the ligand in these tissues

throughout pregnancy (Mayeur *et al*., 2011). Our data suggests that the lower *BDNF* gene expression may be associated with impaired placental development in preeclampsia.

Our findings suggest that low levels of BDNF exist in women with preeclampsia from the 16th week of pregnancy and may be implicated in the improper placental development observed in these women. Further, lower levels of cord BDNF was also observed in preeclampsia which may have long-lasting consequences in their babies.

Like BDNF, NGF too is an important neurotrophin. It was the first neurotrophin to be discovered playing an important role in the nervous system. NGF also plays a role in angiogenesis and may regulate the development of the placenta. The next chapter therefore examines the levels of NGF across gestation in women with preeclampsia. Further, the associations of NGF with BP and birth outcome are also examined.

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Levels of brain derived neurotrophic factors across gestation in women with preeclampsia

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Abstract

Preeclampsia (PE) is a major pregnancy complication of placental origin which leads to adverse pregnancy outcome. Brain derived neurotrophic factor (BDNF) is suggested to promote trophoblast growth and regulate placental and fetal development. This study for the first time examines the levels of maternal plasma BDNF at various time points during gestation, cord plasma and placental BDNF levels and their association with birth outcome in women with PE. Normotensive control (NC) women (n=89) and women with PE (n=61) were followed at three different time points [16-20 weeks (T1), 26-30 weeks (T2) and at delivery (T3)]. Maternal blood at all time points and cord blood was collected. Results indicate that maternal BDNF levels at T1 (p=0.050) and T3 (p=0.025) were lower in women with PE than in NC women. Cord BDNF levels at delivery in women with PE were lower (p=0.032) than those in NC women. Placental BDNF gene expression was also lower (p=0.0082) in women with PE than in NC women. Our data suggests that BDNF plays an important role in the development of the materno-fetal-placental unit during pregnancy. Alteration in the levels of BDNF during pregnancy may be associated with an abnormal development of the placenta resulting in PE.

CHAPTER 4

Gestation Dependent Changes in Nerve Growth Factor Levels in Preeclampsia

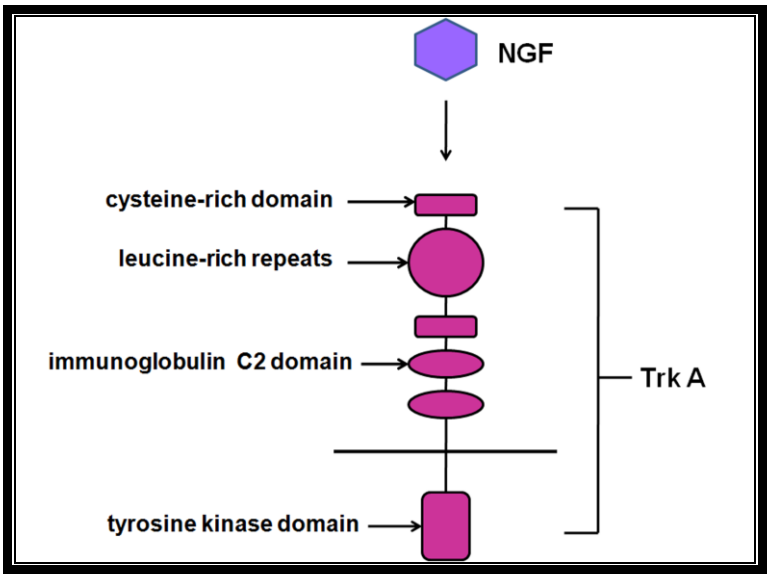
4.1 Introduction

In Chapter 3 we have demonstrated changes in the levels of BDNF from early pregnancy. Studies have also shown that nerve growth factor (NGF) is essential for a successful pregnancy as it constitutes a functional link between the maternal nervous, endocrine and immune system. There are very few studies, which have examined the levels of NGF in preeclampsia. This study for the first time examines the levels of NGF in women with preeclampsia throughout gestation and compares them with normotensive pregnant women.

4.1.1 Nerve Growth Factor

NGF is a member of the neurotrophin family of proteins that was discovered in the 1950's (McKelvey *et al*., 2013) (Fig. 21). NGF has been characterized first in the sensory and autonomic nervous system, then in central nervous, endocrine and immune systems (Aloe *et al*., 2012). The molecular weights of NGFs from human serum, saliva and urine have been found to be 36 kDa (Lipps, 2000). It has a tertiary structure based on a cluster of 3 cystine disulfides and 2 very extended, but distorted beta-hairpins (Bradshaw *et al*., 1994).

Fig. 21: NGF and its Trk A Receptor

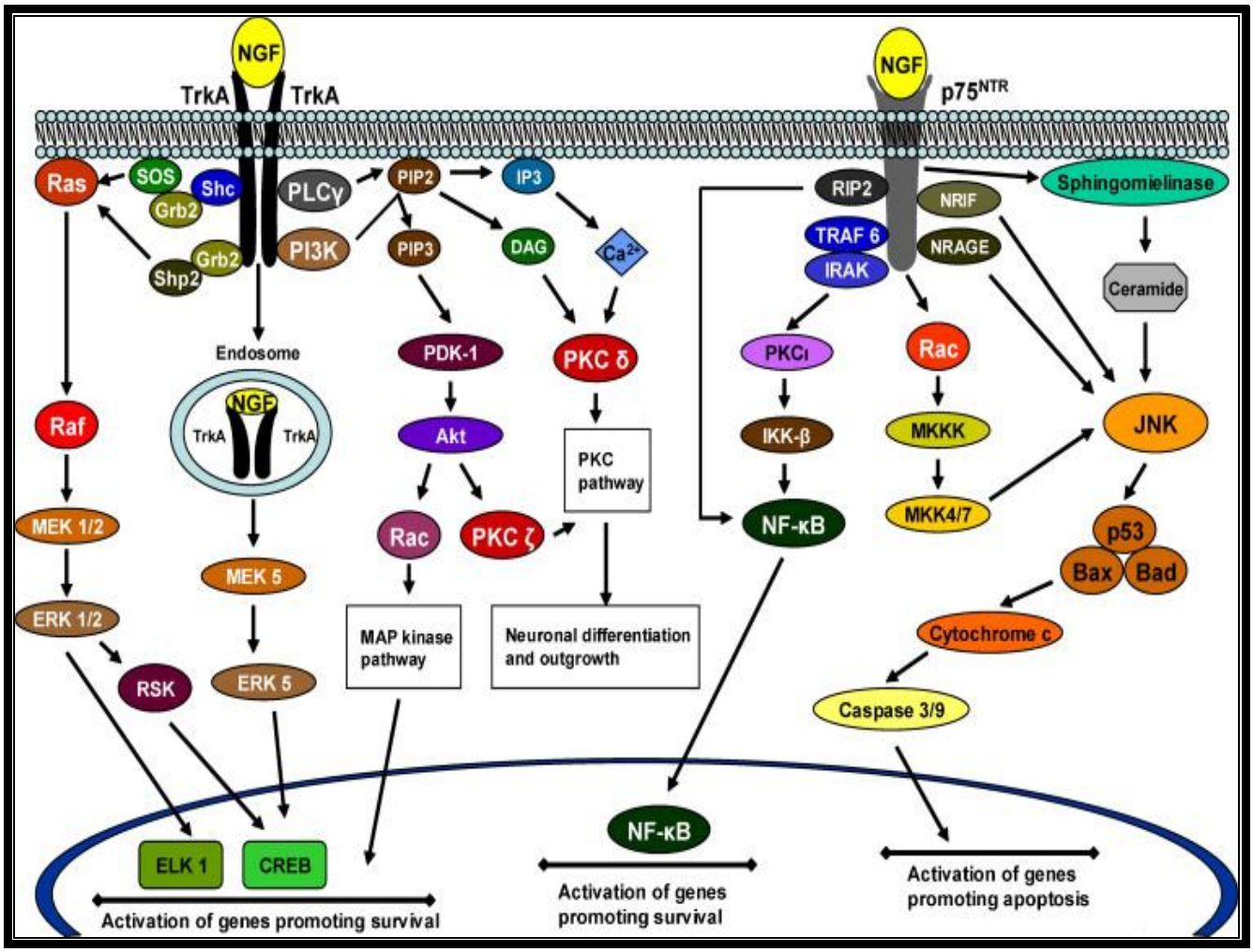


NGF – nerve growth factor; **Trk A** – tropomyosin-related receptor kinase A

Source: Modified from Deinhardt and Jeanneteau, 2012 Protein Phosphorylation in Human Health 7:217-232

NGF is a signaling protein that interacts with specific receptors in autocrine, paracrine and endocrine modes (Rachaneni *et al*., 2013). Its biological activity is mediated by two classes of receptors: (i) p75 neurotrophin receptor (p75NTR), a 75 kDa glycoprotein, belonging to a superfamily of cytokine receptors including TNF receptors, and (ii) Trk A, a transmembrane tyrosine kinase of 140 kDa (Fiore *et al*., 2009) (Fig. 22). Different intracytoplasmic signaling pathways that may be activated by binding of NGF to Trk A receptor include phosphatidylinositol-3-kinase (PI3K) and mitogenic activated protein kinase (MAPK) to promote cell survival and proliferation and activation of the phospholipase type Cγ (PLCγ) pathway. This results in the production of diacylglycerol (DAG) and inositol triphosphate (IP3), culminates in the release of calcium from the intracytoplasmic cellular stocks (Chaves *et al*., 2013).

Fig. 22: NGF- Trk A Signalling Pathways



Akt - serine/threonine kinase; **CREB -** cAMP response-element binding protein; **DAG -** diacylglycerol; **ELK 1-** E twenty-six (ETS)-like transcription factor 1; **ERK -** extracellular signal regulated kinase; **IKK -** NF-κB-binding cofactor kinase; **IP3 -** inositol trisphosphate; **IRAK -** interleukin-1β receptor-associated kinase; **JNK -** c-Jun N-terminal kinase; **MEK –** mitogen activated protein kinase/ERK kinase; **NF-κB -** nuclear factor-κB; **NGF –** nerve growth factor; **NRAGE -** neurotrophin receptor-mediated melanoma-associated antigens homolog; **NRIF -** neurotrophin receptor interacting factor; **p75NTR –** p75 neurotrophin receptor; **PDK1 -** 3-phosphoinositide-dependent protein kinase 1; **PI3K –** phosphotidylinositol 3-kinase; **PKC -** protein kinase C; **PIP -** phosphatidylinositol-bis-phosphate; **PLCγ –** phospholipase Cγ; **Rac, Raf, Ras -** rho-associated kinases; **RIP2 -** receptor interacting protein 2; **RSK -** ribosomal S6 kinase; **Shc -** Src homology and collagen related molecules; **SOS -** son of sevenless proteins; **TNAF 6 -** tumour necrosis factor receptor-associated factor 6; **Trk A -** tropomyosin-related receptor kinase A

Source: Modified from Pollack and Harper, 2002 Drug News Perspect, 15:268

4.1.2 Functions of NGF in the Nervous System

NGF was discovered as a molecule that stimulates the survival and maturation of developing sensory neurons in the peripheral nervous system (PNS) and has later

been shown to protect adult neurons in the degenerating mammalian brain (Roberti *et al*., 2014; Manni *et al*., 2013). Additionally, NGF is known to have trophic and differentiating activity on several populations of cholinergic neurons of the CNS (Roberti *et al*., 2014). NGF ensures the maintenance of phenotypic and functional characteristic of several populations of neurons as well as immune cells (Aloe *et al*., 2012). It has important functions in the maintenance of viability and proliferation of neuronal and non-neuronal cells (Chaves *et al*., 2013). Recent studies have recognized the role of NGF beyond nerve cells and the peripheral PNS and CNS and that topical application of NGF give a protective effect on corneal ulcer and glaucoma (Manni *et al*., 2013).

4.1.3 NGF and Neurodevelopment

Neurodevelopment is coordinated by a series of growth factors including NGF which are involved in neuritogenesis (Li *et al*., 2014). NGF is related to the growth and differentiation of nerve cells, as well as to a decline in cognitive function (Bae *et al*., 2014). Enhancement in NGF levels after brain injury is a part of neuronal recovery process (Dinçel *et al*., 2013). NGF concentrations in serum and cerebrospinal fluid (CSF) may be enhanced in various cerebral pathologies such as hypoxia, ischaemia, trauma, seizures, neuroimmunological diseases and intracranial hypertension syndrome (Gioiosa *et al*., 2009; Sofroniew *et al*., 2001; Dixon *et al*., 1997). A recent study has shown that maternal psychological and biological factors are associated with infant motor development and are mediated by changes in NGF levels (Pinheiro *et al*., 2014).

4.1.4 NGF and Placenta

Human placental tissue contains a significant amount of NGF (Purcell and Atterwill, 1994). Reports indicate that NGF is expressed, localized and active in the trophoblast, amnion/chorion and maternal decidua of the placenta both in early gestation and at term (Toti *et al*., 2006; Marvin *et al*., 2002). Growth factors such as NGF, VEGF, produced by nerves and blood vessels may contribute to the process of normal placentation and may be compromised in pregnancy complications (Quinn, 2005).

4.1.5 NGF and Angiogenesis

NGF has historically been implicated in several functions of the nervous system, however, it can also regulate hypertension (Hennigan *et al*., 2009; Supowit *et al*., 2001) and elicit cardiovascular actions, including angiogenesis during foetal and neonatal stages (Meloni *et al*., 2010; Cabrera-Vásquez *et al*., 2009). Blood vessel and nerve growth are linked by common pathways that involve the release of proangiogenic factors, such as vascular endothelial growth factor, β-NGF and neuropeptides (Mapp and Walsh, 2012). Every endothelial cell destined for vessel formation is equipped with receptors for angiogenic peptides such as NGF (Gacche and Meshram, 2013). NGF has been shown to have an influence on the functional activity of the cardiovascular system and can regulate the functional state of endothelial and vascular smooth muscle cells and cardiomyocytes (Kryzhanovskiĭ and Vititnova, 2011).

4.1.6 NGF and Preeclampsia

Recent studies have revealed that preeclampsia appears to originate in placenta and is characterized by widespread maternal endothelial dysfunction (Petla *et al*., 2013). Abnormal placentation and reduced blood flow in preeclampsia 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 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 preeclampsia (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 preeclampsia due to improper development of placenta.

NGF plays an important role in the regulation of growth, survival and differentiation of neurons in both the CNS and PNS (Bibel and Barde, 2000). Although NGF has historically been implicated in several functions of the nervous system, it can also regulate hypertension (Hennigan *et al*., 2009; Supowit *et al*., 2001) and elicit cardiovascular actions, including angiogenesis during foetal and neonatal stages (Meloni *et al*., 2010; Cabrera-Vásquez *et al*., 2009). It has been reported that NGF is mandatory for the success of pregnancy as it constitutes a functional link between the nervous, endocrine and immune system translating environmental or endocrine signals (Tomettan *et al*., 2005). It has been reported that NGF levels increase as the trimester progresses in monkeys (Neubert *et al*., 2014). A recent study suggests that a local threshold of NGF expression is necessary to ensure maternal

tolerance in healthy pregnancies, but when surpassed may result in foetal rejection due to exacerbated inflammation (Frank *et al*., 2014).

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 preeclampsia. Xia *et al.* (2011) have shown reduced NGF levels in amniotic fluid specimens were obtained during amniocentesis or caesarean section (Xia *et al*., 2011).

Our earlier cross sectional study on women with preeclampsia has shown altered NGF levels as compared to normotensive women (Kilari *et al*., 2011). Although preeclampsia is characteristically diagnosed in the last third of pregnancy, it is evident that many of these pathophysiological changes can be detected long before clinically evident disease (Roberts and Bell, 2013). Further, infants born to women with preeclampsia are at increased risk for cardiovascular and neurodevelopmental disorders (Akcakus *et al*., 2010; Silveira *et al*., 2007). Hence there is a need to study the maternal and cord NGF levels across the pregnancy to study the mechanism and severity of preeclampsia.

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

4.2 Materials and Methods

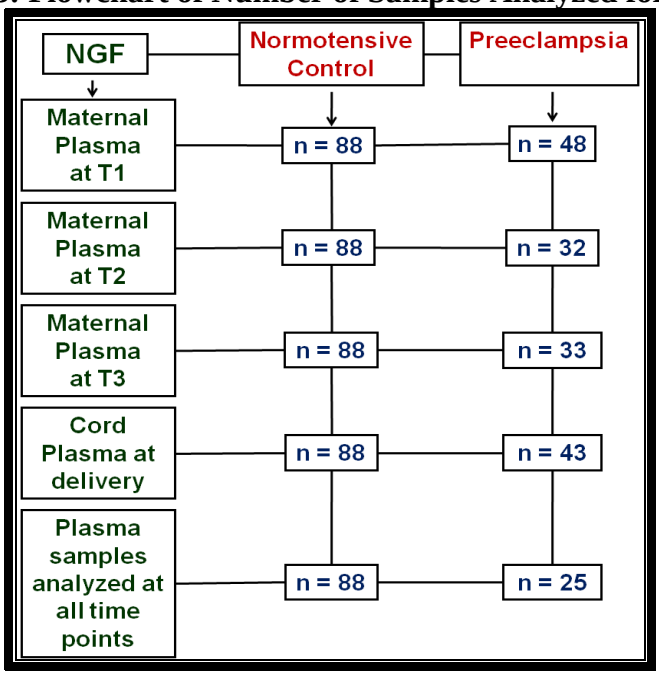
4.2.1 Participants

The recruitment of study population and the inclusion and exclusion criteria are as mentioned in Chapter 3 (Section 3.2.1). Neonatal growth measures were recorded as mentioned in Chapter 3 (Section 3.2.1).

4.2.2 Power of the Study

Till date there are no studies examining plasma NGF levels at various time points across gestation. This is the first longitudinal study of NGF in women with preeclampsia. Therefore we calculated the power of the study based on our earlier cross sectional study on NGF levels in preeclampsia (Kilari *et al*., 2011). Based on results from this study the Power and Sample Size Calculation version 3.0.43 software was used to calculate sample size of the current study. In our earlier study, NGF levels in NC (316.3 ± 109.7 pg/ml) and preeclampsia (257.6 ± 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 preeclampsia 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 (Fig. 23).

Fig. 23: Flowchart of Number of Samples Analyzed for NGF



NGF – nerve growth factor; **n** – number; **T1** - 16-20 weeks of gestation; **T2** - 26-30 weeks of gestation; **T3** - at delivery

4.2.3 Sample Collection, Processing and Storage

Blood sample collection was as described in Chapter 2 (Section 2.2.2) while placental sample collection was as described in Chapter 3 (Section 3.2.2)

4.2.4 NGF Assay

NGF levels were measured in maternal and cord blood plasma samples using the NGF Emax Immuno Assay System Promega kit (Zettler *et al*., 1996). Briefly, the NGF Emax® Immunoassay system is designed for the sensitive and specific detection of NGF in the antibody sandwich format. In this format, flat bottom 96-well plates are coated with anti-NGF polyclonal antibody (pAb) which binds soluble NGF. The captured NGF is bound by a second monoclonal antibody (mAb). After washing, the amount of specifically bound mAb is then detected using a species-specific antibody conjugated to horse radish peroxidase (HRP) as a tertiary reactant.

The unbound conjugate is removed by washing and following incubation with a chromogenic substrate. The colour change is measured by a spectrophotometer using an ELISA plate reader. The amount of NGF in the test solutions is proportional to the colour generated in the oxidation–reduction reactions. NGF concentrations were expressed as pg/ml. We have reported this method in our earlier studies (Dhobale *et al*., 2012b; Kilari *et al*., 2011).

4.2.5 Extraction of Total RNA, cDNA Synthesis and Quantitative Real-Time (RT)-PCR Assays

Extraction of total RNA, cDNA synthesis and mRNA expression of *NGF* and *GAPDH* genes from 22 NC and 24 preeclamptic placental samples was performed. The detailed method has been described by us earlier in Chapter 3 (Section 3.2.4) (D'Souza *et al*., 2014). The following TaqMan® Gene Expression Assays (Applied Biosystems) were used in this study: *GAPDH* (Hs99999905_m1); *NGF* (Hs01113193_m1). The *NGF* mRNA levels are expressed as mean ± SE.

4.2.6 Statistical Analysis

Continuous variables are reported as mean ± S.D. and discrete variables as percent (%). The data were checked for normal distribution by testing for skewness and kurtosis and NGF values were found normally distributed. Differences in maternal and neonatal characteristics between preeclampsia and NC women were assessed using Student's t test for continuous variables and chi-square tests for categorical data. Pearson's correlation was used to examine the associations between NGF levels and maternal age, body mass index (BMI), gestational age, parity, socioeconomic status (SES), education and income as they have been shown to affect

NGF levels (Kumar *et al*., 2012; Hackman *et al*., 2010; López-Valladares *et al*., 2010; Bulló *et al*., 2007; Marx *et al*., 1999). NGF was correlated with gestational age, BMI and SES and hence NGF levels were adjusted for these variables using multiple linear regression.

Associations of NGF with BP and neonatal growth measures were analyzed after adjusting for gestational age, BMI and SES. Results corresponding to p-values lower than 0.05 (5%) are described as significant and reported. The data were analyzed using the SPSS/PC+ package (Version 20.0, Chicago, IL, USA).

4.3 Results

4.3.1 Maternal and Neonatal Characteristics

The maternal BMI at all time points in women with preeclampsia (T1, $p<0.05$; T2, $p<0.05$; T3, $p<0.01$) was higher than NC women. Maternal systolic at T1, T2 and T3 and diastolic BP at T1, T2 and T3 ($p<0.01$ for all) in women with preeclampsia was higher than NC women. The percentage of nulliparous women was higher in the preeclampsia group as compared to the NC group (65% vs. 35%, $p = 0.038$). Parity was found to be different between groups. There was no difference in education and income between the groups (Table 5).

Table 5: Maternal Characteristics

	NC (n=88)	PE (n=48)
Age in years	27 ± 4	26 ± 5
BMI in kg/m2 **T1** **T2** **T3**	 22 ± 4 24 ± 4 26 ± 4	 24 ± 5* 27 ± 5* 29 ± 5**
Gestation in weeks (range) **T1** **T2** **T3**	 18 ± 2 29 ± 2 39 ± 1	 17 ± 2 30 ± 2* 37 ± 3**
Sys BP in mmHg **T1** **T2** **T3**	 110 ± 9 112 ± 8 120 ± 9	 118 ± 11** 122 ± 13** 145 ± 15**
Dias BP in mmHg **T1** **T2** **T3**	 72 ± 7 70 ± 7 77 ± 6	 75 ± 8* 77 ± 8** 95 ± 12**
Education in n (%) **lower** **higher** **graduation** **post graduation**	 29 10 36 25	 24 15 42 19
Parity in n (%) **nulliparous** **multiparous**	 47 53	 65 35
Income in INR (range)	3000 - 75000	5000-75000

Values are expressed as Mean ± S.D.; *p<0.05, **p<0.01 as compared to NC; **BMI -** body mass index; **Dias BP -** diastolic blood pressure; **n –** number; **NC -** normotensive control; **PE -** preeclampsia; **Sys BP -** systolic blood pressure; **T1 -** 16-20 weeks of gestation; **T2 -** 26-30 weeks of gestation; **T3 -** at delivery

Table 6: Neonatal Characteristics at Birth

	NC (n=88)	PE (n=48)
Weight (kg)	2.9 ± 0.3	2.8 ± 0.6*
Length (cm)	48.1 ± 2.9	47.6 ± 2.4
Head Circumference (cm)	33.9 ± 1.3	33.3 ± 1.3*
Chest Circumference (cm)	32.2 ± 1.7	31.5 ± 1.8*

Values are expressed as Mean ± S.D.; *p<0.05, as compared to NC.
NC – normotensive control; **PE -** preeclampsia

Neonatal characteristics show that birth weight (p<0.05) and head circumference (p<0.05) was lower in preeclampsia than in the NC group (Table 6).

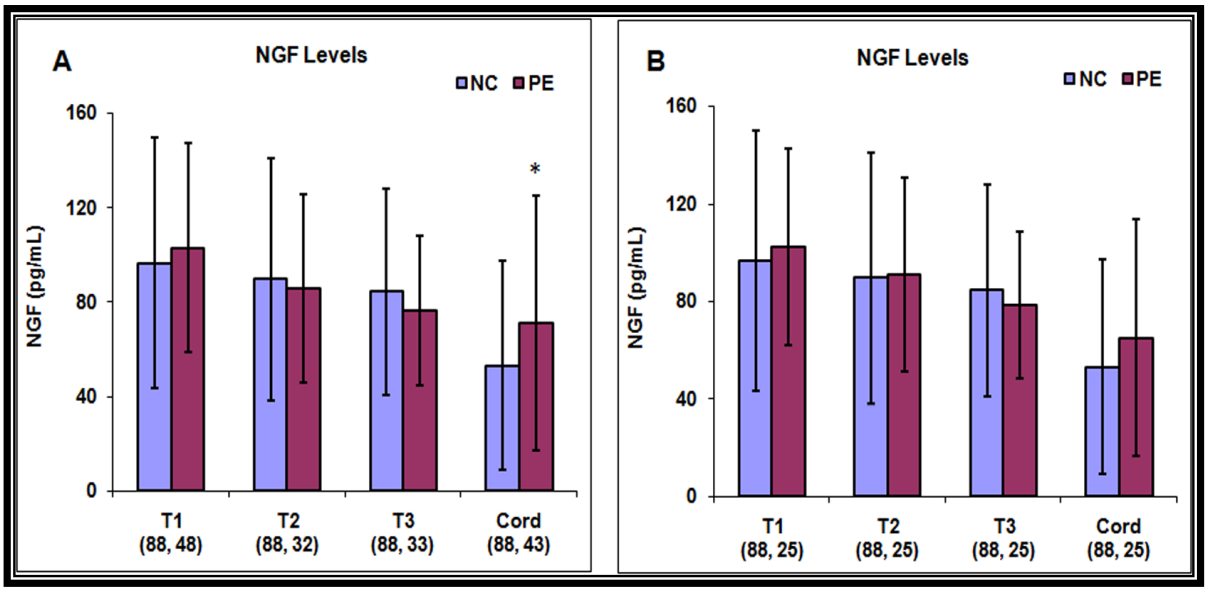
4.3.2 Maternal NGF levels

Maternal NGF levels at T1 (NC: 96 ± 53 pg/mL; PE: 103 ± 44 pg/mL), T2 (NC: 90 ± 51pg/mL; PE: 86 ± 40 pg/mL) and T3 (NC: 84 ± 44 pg/mL; PE: 76 ± 32 pg/mL) were comparable in women with preeclampsia as compared to NC women (Fig. 24).

4.3.3 Cord NGF levels

Cord NGF levels were higher in preeclampsia (70 ± 53 pg/mL) (p<0.05) as compared to the NC group (53 ± 44 pg/mL) when compared between all samples (Fig 24A). However, cord NGF levels showed a trend but were not significant in samples obtained at all time points (Fig 24B).

Fig. 24: Maternal and Cord NGF Levels in Women with Preeclampsia as Compared to Normotensive Control Women

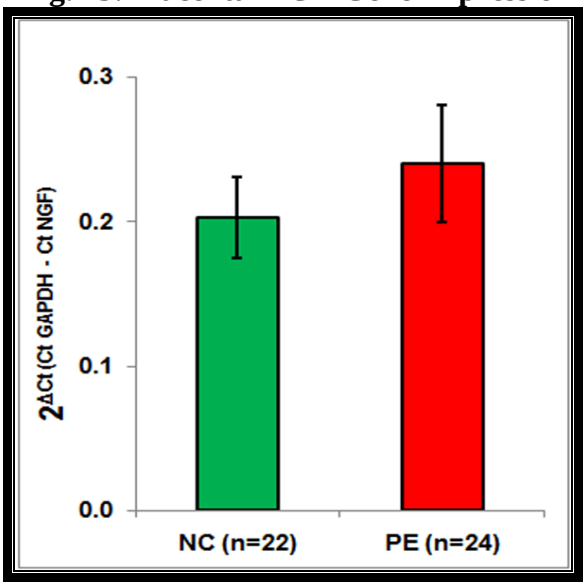


Values are expressed as Mean ± SD; *p<0.05 as compared to NC. **A -** all samples; **B -** all timepoints; **NC -** normotensive control; **NGF –** nerve growth factor; **PE -** preeclampsia; **T1 -** 16 to 20 weeks of gestation; **T2 -** 26 to 30 weeks of gestation; **T3 -** at delivery

4.3.4 Placental *NGF* Gene Expression

Placental *NGF* gene expression was higher although not significant in women with preeclampsia as compared to NC women (Fig. 25).

Fig. 25: Placental *NGF* Gene Expression



Values are expressed as Mean ± S.E.; **NC -** normotensive control; **PE** – preeclampsia

4.3.5 Association between Maternal NGF Levels and Maternal Blood Pressure at T1 and T2

There was a negative association between maternal NGF levels and maternal diastolic BP at T1 ($r = -0.471$, $p = 0.027$, $n = 25$) and T2 ($r = -0.412$, $p = 0.063$, $n = 25$) in women with preeclampsia.

4.3.6 Association between Cord NGF Levels and Neonatal Growth Measures

There was a positive association between cord NGF levels and baby head circumference ($r = 0.218$, $p = 0.041$, $n = 92$) and chest circumference ($r = 0.218$, $p = 0.041$, $n = 92$) in the whole cohort.

4.4 Discussion

This study reports novel findings 1) Maternal NGF levels in women with preeclampsia were comparable to NC women at all time points 2) Cord NGF levels at delivery were higher in women with preeclampsia as compared to NC women 3) A negative association was observed between maternal diastolic BP and NGF levels at T1 in women with preeclampsia 4) There was a positive association between cord NGF levels and baby head and chest circumference in the whole cohort.

4.4.1 Higher Cord NGF Levels

In this study, maternal NGF levels in women who develop preeclampsia were comparable to NC women. In contrast, there was an increase in cord levels in women with preeclampsia. NGF is of major importance in prenatal and postnatal brain development, due to its neuroprotective action. One study has shown lower circulating levels of NGF in mothers delivering intrauterine growth restricted babies

(IUGR) as compared to mothers delivering appropriate for gestational age (AGA) babies (Malamitsi- Puchner *et al*., 2007). Studies have shown that loss of neural connections through the process of partial uterine denervation may cause reduced foetal growth without the maternal circulatory changes of preeclampsia (Quinn, 2005).

Studies suggest that NGF is involved in angiogenesis (Park *et al*., 2007) and acts in a pleiotropic manner at both neuronal and vascular levels (Nico *et al*., 2008). Our earlier study in women with preeclampsia has shown altered levels of angiogenic factors (Kulkarni *et al*., 2010). Studies suggest that there is a cross talk between cardiovascular and nervous systems. Growth factors like VEGF and NGF have been implicated in both neurodegenerative and vascular diseases (Lazarovici *et al*., 2006). It has been reported that physiological actions of NGF and VEGF are mediated by receptors (Trk A for NGF and VEGFR-2 for VEGF) which activate a complex and integrated network of signaling pathways in neurons and endothelial cells (Lazarovici *et al*., 2006).

In the current study, although there was no change in maternal NGF levels in women with preeclampsia there was an increase in cord NGF levels. Further, placental *NGF* mRNA levels were higher though not statistically significant possibly due to the small sample size in women with preeclampsia as compared to NC women. Studies from our department have earlier reported increased oxidative stress in women with preeclampsia (Mehendale *et al*., 2008). This increase in the expression of NGF may possibly lead to an increase in cord NGF levels. Our findings suggest that this may be a compensatory mechanism to protect the developing foetus from adverse outcomes.

Studies from our department have earlier reported that maternal NGF levels are lower in women with preeclampsia delivering low birth weight babies as compared to normotensive women delivering low birth weight babies (Kilari *et al*., 2011). Our departmental study has also reported lower cord NGF levels in preterm pregnancy (Dhobale *et al.,* 2012b). Future studies need to understand the mechanisms influencing NGF levels in pregnancy.

4.4.2 Negative Association between Maternal NGF and Maternal Diastolic Blood Pressure at T1

A negative association was observed between maternal diastolic BP and NGF levels at T1 in women with preeclampsia. Neurotrophins are involved in sympathetic hyperinnervation and progression of systemic hypertension (Ricci *et al*., 2004). Recent studies indicate that a reduction in maternal/adult NGF level plays an important role in reducing/increasing metabolic syndrome (Histova, 2013). Reports indicate that an enhanced excitation of the sympathetic nervous system by NGF may be a common mechanism underlying hypertension (Chen *et al*., 2011). NGF released during and following stress may serve to prevent possible deficits and/or damage linked to sympathetic and cardiovascular activation (Manni *et al*., 2008). However the exact mechanisms associated with the NGF and BP need additional research.

4.4.3 Positive Association between Cord NGF and Baby Head Circumference

There was a positive association between cord NGF levels and baby head circumference in the whole cohort. NGF plays a role in neurodevelopment and is reported to be altered in several neurodevelopmental disorders such as schizophrenia and attention deficit hyperactivity disorder (ADHD) (Syed *et al*., 2007; Shoval and

Weizman, 2005). Our results are similar to an earlier study that reports a positive correlation between maternal NGF levels and birth weight in IUGR infants (Malamitsi-Puchner *et al*., 2007). NGF levels are reported to correlate with customized centiles and birth weights of infants (Malamitsi and Puchner *et al*., 2007). Reports indicate that Beta-NGF levels show developmental changes and may be used to assess NGF utilization under normal and pathologic conditions (Haddad *et al*., 1994).

Studies suggest that maladaptive or repeated activation of NGF, early during postnatal life, may influence stress sensitivity at adulthood and increase vulnerability for stress-related psychopathology (Cirulli *et al*., 2009). During early developmental phases, experiences such as maternal deprivation or exposure to an enriched environment are known to markedly affect NGF levels (Branchi *et al*., 2004). Further, studies in rats have suggested that the offspring that were exposed to increased maternal care had reduced methylation of transcription factor NGF-inducible A, increased expression of hippocampal glucocorticoid receptor and a modest HPA responses to stress (Champagne, 2008; Weaver, 2007). Environment enrichment is reported to upregulate the expression of cellular signals that are involved in activity dependent synapse formation involving neurotrophins such as NGF (van Praag *et al*., 2000). Preeclampsia is known to be a stressful condition and increased oxidative stress in women with preeclampsia has been reported by us earlier (Mehendale *et al*., 2008).

Thus, our data suggests that neurotrophins may influence pregnancy outcome possibly by regulating angiogenesis. Studies from our department have earlier reported lower placental LCPUFA levels and altered fatty acid metabolism in preeclampsia (Wadhwani *et al*, 2014; Kulkarni *et al*, 2010). Our department has also

demonstrated that DHA influences global methylation levels in the placenta (Kulkarni *et al*, 2011). It is therefore likely that the expression of neurotrophins may be influenced by prenatal LCPUFA metabolism. Future studies need to examine the role of DHA in epigenetically regulating neurotrophins on a larger sample size.

The current longitudinal study for the first time reports maternal and cord NGF levels in women with preeclampsia and compares them with NC women. Studies have shown that oxidative stress can regulate neurotrophins such as NGF and BDNF. Our data suggests that higher cord NGF levels may possibly be a response to higher oxidative stress observed in preeclampsia.

The next chapter therefore examines oxidative stress markers throughout gestation in women with preeclampsia and also examines their association with neurotrophins such as BDNF and NGF.

CHAPTER 5

Gestation Dependent Changes in Oxidative Stress Markers and their Association with Neurotrophins in Preeclampsia

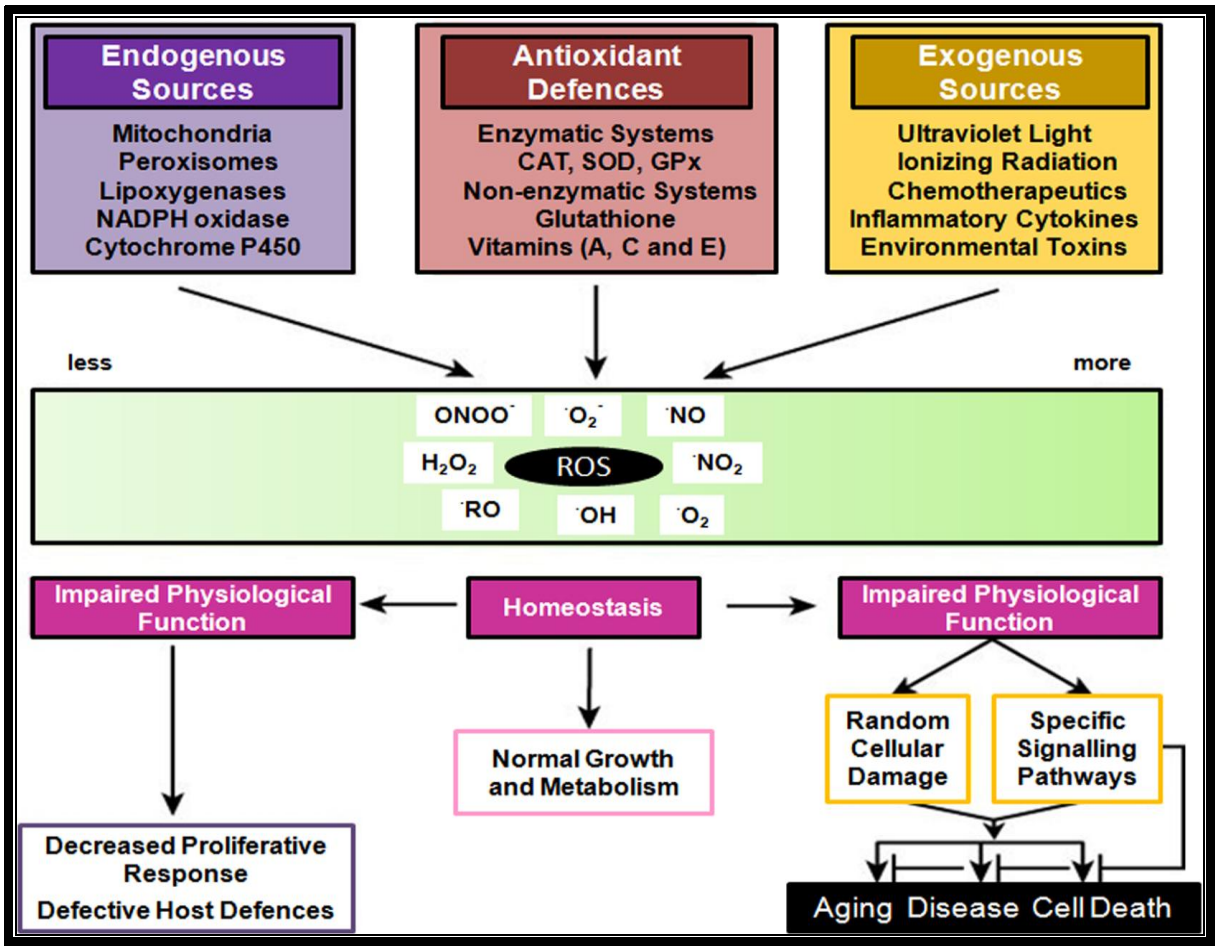
5.1 Introduction

Oxidative stress is defined as a process resulting from an imbalance between production of reactive oxygen species (ROS) and antioxidant capacity that damages cell structures, including lipids, proteins and DNA. **Reports indicate that oxidative stress can influence neurotrophin levels. This study therefore examines oxidative stress levels across gestation in women with preeclampsia and compares it with normotensive women. Their association with neurotrophins (BDNF and NGF), blood pressure and birth outcome is also examined.**

5.1.1 Free Radicals and Reactive Oxygen Species

A molecule with one or more unpaired electron in its outer shell is called a free radical. Once formed free radicals participate in several reactions yielding ROS (Ortiz *et al.*, 2008). Free radicals such as superoxide, nitric oxide and hydroxyl radicals and ROS such as hydrogen peroxide, peroxynitrite and hypochlorous acid are produced in the body, primarily as a result of aerobic metabolism (Fang *et al*., 2002). They play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body (Pham-Huy *et al*., 2008) (Fig. 26). A cross-talk of molecular oxygen, nitric oxide and superoxide radicals regulates the circulation, energy metabolism, apoptosis and functions as a major defense system against pathogens (Inoue *et al*., 2003). In contrast, imbalance between ROS production and antioxidant systems induces oxidative stress (Al-Gubory *et al*., 2010). Oxidative stress may be detrimental in acquired immunity by activation of nuclear factor kappa B, which governs gene expression involving various cytokines, chemokines and cell adhesion molecules (Knight, 2000).

Fig. 26: Dual Role of Reactive Oxygen Species



CAT - catalase; **GPx** - glutathione peroxidase; **H_2O_2** – hydrogen peroxide; **O_2** - oxygen; **O_2^-** - superoxide anion, **OH^-** - hydroxy ion; **$ONOO^-$** - peroxy nitrite **NO** - nitric oxide; **ROS** - reactive oxygen species; **SOD** - superoxide dismutase

Source: Modified from Finkel and Holbrook, 2000 Nature 408:239-47.

5.1.2 Biological Generation of ROS

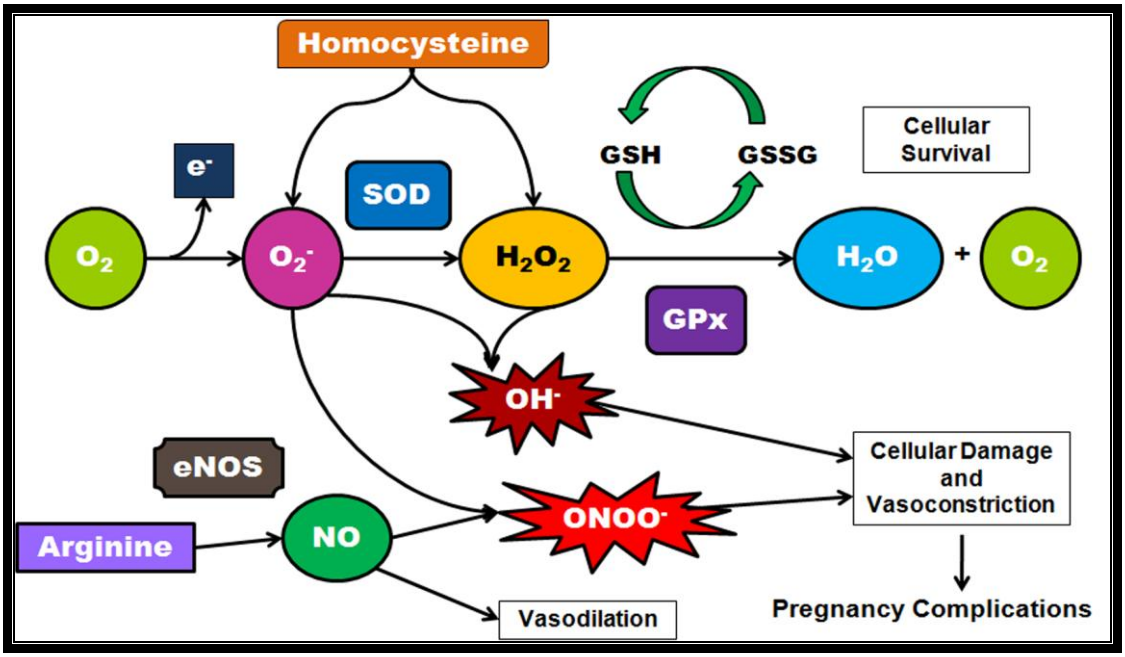
ROS are a family of molecules that are continuously generated, transformed and consumed in all living organisms as a consequence of aerobic life (Dickinson and Chang, 2011). The main routes of their formation in living organisms include interaction of physical agents with cells and organisms, auto-oxidation of biochemical intermediates, xenobiotics and biochemical synthesis for defence (Bartosz, 2003). Components of tobacco smoke, air pollutants, the metabolism of certain chemicals and exposure to radiation lead to the origin of exogenous free radicals (Machlin

and Bendich, 1987). Free radicals can originate endogenously from normal metabolic enzymatic reactions involved in respiratory chain, phagocytosis, prostaglandin synthesis and in the cytochrome P-450 system (Lobo *et al*., 2010). ROS such as superoxide, hydrogen peroxide, single oxygen and lipid peroxides are derived from many sources including mitochondria, xanthine oxidase, uncoupled nitric oxide synthases and NADPH oxidase (Mueller *et al*., 2005).

5.1.2.1 Homocysteine

Homocysteine, which is biosynthesized from methionine in the one carbon cycle is also known to be associated with oxidative stress. It has been suggested that thiol group of homocysteine can rapidly auto-oxidize in circulation in the presence of ceruloplasmin, the major copper binding protein in plasma, to form homocysteine and hydrogen peroxide (H_2O_2), thereby generating oxidative stress (Starkbaum and Harlan, 1986) (Fig. 27).

Fig. 27: Mechanism of Oxidative Stress Leading to Pregnancy Complications



CAT - catalase; **eNOS** - endothelial nitric oxide synthase; **GPx** - glutathione peroxidase; **GSH** - oxidised glutathione; **GSSG** - reduced glutathione, **H_2O** - water; **O_2** - oxygen; **O_2^-** - superoxide anion, **OH^-** - hydroxy ion; **NO** - Nitric oxide; **$ONOO^-$** - peroxy nitrite; **SOD** - superoxide dismutase

5.1.3 ROS and Cell Damage

Excessive production of ROS can cause progressive oxidative damage and ultimately cell death (Sharma *et al*., 2012). Free radicals and ROS are highly reactive molecules that can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA and critical sulfhydryl bonds in proteins (Machlin and Bendich, 1987). Specific ROS can lead to different molecular cell death mechanisms such as necrosis and apoptosis (Valencia and Morán, 2004). The trigger for diseases may be diverse but inflammation and/or oxidative stress may be basic mechanisms increasing the severity or complicating the condition of the disease (Chaudhari *et al*., 2014).

5.1.4 Products of Oxidative Damage

Oxidative stress and oxidative modification of biomolecules are involved in a number of physiological and pathophysiological processes such as aging, artheroscleosis, inflammation, carcinogenesis and drug toxicity (Lobo *et al*., 2010). Overproduction of ROS results in oxidative stress and is an important mediator of damage to cell structures, including lipids and membranes, proteins and DNA (Valko *et al*., 2007).

5.1.4.1 Lipid Peroxidation

ROS can react with the PUFA of lipid membranes and induce lipid peroxidation (Barrera, 2012). Lipid peroxidation is known to be involved in endothelial cell dysfunction in preeclampsia (Gupta *et al*., 2005). Phosphatidylcholine hydroperoxide is the primary product of lipid peroxidation, which undergoes non-enzymatic reactions, leading to the formation of 4-hydroxynonenal (HNE) and malondialdehyde (MDA), secondary products of lipid peroxidation (Uchida, 2003; Esterbauer *et al*., 1991). Lipid peroxidation products are known to regulate gene expression, signalling and activate receptors (Noguchi *et al*., 2008; Niki, 2008).

5.1.4.2 Protein Oxidation

Proteins can be oxidatively modified in three ways: oxidative modification of specific amino acid, free radical mediated peptide cleavage and formation of protein cross-linkage due to reaction with lipid peroxidation products (Lobo *et al*., 2010). Protein containing amino acids such as methionine, cysteine, arginine, and histidine are the most vulnerable to oxidation (Freeman and Crapo, 1982). One of the hallmarks of severe oxidative stress is the accumulation of oxidized proteins, which

tend to form high molecular weight aggregates (Bader and Grune, 2006). Protein aggregation occurs if protein repair and degradative systems are unable to act upon oxidized proteins and restore cellular function (Squier, 2001).

5.1.4.3 DNA Oxidation

ROS can cause severe damage to DNA which can determine cell fate through cell cycle arrest and DNA repair or the activation of apoptotic pathways (Barzilai and Yamamoto, 2004). DNA bases are vulnerable to oxidative stress damage involving hydroxylation, protein carbonylation and nitration (Lovell and Markesbery, 2007). The main oxidation product in cellular DNA is 8-oxo-7, 8-dihydroguanine (Cadet *et al*., 2014). Hydroxymethylcytosine, formylcytosine, and carboxylcytosine are products generated by oxidation of methylated DNA (Delatte *et al*., 2014). Oxidatively damaged DNA has been associated with aging, cancer and some degenerative diseases (Chao *et al*., 2013).

5.1.5 Antioxidants

The human body has several mechanisms to counteract oxidative stress one of which is by producing antioxidants, which are either naturally produced *in situ* (endogenous), or externally supplied through foods and/or supplements (exogenous) (Pham-Huy *et al*., 2008). Antioxidants including antioxidant enzymes and antioxidant metabolites exert synergistic actions in scavenging free radicals and ROS (Fang *et al*., 2002). Antioxidants act as safeguard against the accumulation of ROS and their elimination from the system (Rajendran *et al*., 2014). However their target selectivity and antioxidant effectiveness vary for different oxidants (Winterbourn, 2008).

5.1.5.1 Antioxidant Enzymes

Antioxidant enzymes play a major role in ROS scavenging and changes of their expression or/and activity are reported to be associated with several disorders (Tokarz *et al*., 2013; Fujita *et al*., 2012). In order to overcome the oxidative damage, there are some protective signalling pathways related to transcriptional upregulation of antioxidant enzymes (Fujita *et al*., 2012). Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST), glutathione reductase (GR) and glutathione peroxidases (GPx) (Fang *et al*., 2002).

Superoxide Dismutase

SODs were discovered by McCord and Fridovich between 1968–1969 (Buettner, 2011). They are major antioxidant enzymes for the removal of superoxide radicals in different subcellular compartments (Huang *et al*., 2012). SOD is the first line of defence against oxidative stress under physiological and pathological conditions (Batinić-Haberle *et al*., 2010). They catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Lobo *et al*., 2010). SODs consist of three isoforms in mammals: the cytoplasmic Cu/ZnSOD (SOD1), the mitochondrial MnSOD (SOD2), and the extracellular Cu/ZnSOD (SOD3) (Fukai and Ushio-Fukai, 2011). The human gene for SOD1 has been localized to the 21q22.1 region of chromosome 21 that encodes the 32kDa homodimer (Levanon *et al*., 1985).

Glutathione Peroxidase

Cellular GPx was the first enzyme recognized as a selenoprotein. In GPx selenium (Se) was found in the form of selenocysteine. The enzyme is a tetrameric protein and is composed of four apparently identical subunits (Zachara, 1992). GPx

protects the organism from oxidative damage by catalyzing the reduction of harmful hydroperoxides with thiol cofactors (Pieczyńska and Grajeta, 2015; Bhabak and Mugesh, 2010). The selenocysteine residue at the active site of this enzyme forms a "catalytic triad" with tryptophan and glutamine, which activates the selenium moiety for an efficient reduction of peroxides (Bhabak and Mugesh, 2010). GPx removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized GSH (Lobo *et al.*, 2010). A total of eight mammalian GPx are classified according to their functions in the system (Ramming and Appenzeller-Herzog, 2013). GPx are involved in signalling cascades, e.g. in the insulin signalling pathway by GPx1; GPx2 plays a dual role in carcinogenesis depending on the mode of initiation and cancer stage; GPx3 is membrane associated possibly explaining a peroxidatic function despite low plasma concentrations of GSH; GPx4 has novel roles in the regulation of apoptosis and, together with GPx5, in male fertility. Functions of GPx6 are still unknown, and the proposed involvement of GPx7 and GPx8 in protein folding awaits elucidation (Brigelius-Flohé and Maiorino, 2013).

Endothelial Nitric Oxide Synthase

Nitric oxide (NO·) is an important protective molecule in the vasculature, and endothelial NO· synthase (eNOS) is responsible for most of the vascular NO· produced (Förstermann and Münzel, 2006). eNOS couples with essential cofactor (6R)-5,6,7,8-tetrahydro-L-biopterin (BH_4) and catalyzes the conversion of arginine, oxygen and NADPH to NO· and citrulline (Solomonson *et al*., 2003). Cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes mellitus, or chronic smoking stimulate the production of reactive oxygen species in the vascular wall (Tousoulis *et al*., 2011). Superoxide reacts avidly with vascular NO· to form

peroxynitrite which is known to accumulate in placental tissues in pregnancy complications such as preeclampsia (Matsubara *et al*., 2015). Peroxynitrite oxidizes BH_4 promoting superoxide production by eNOS (Förstermann and Münzel, 2006). It is believed that new information about redox regulation of eNOS may point to ways of controlling oxidative stress in the vasculature (Hoang *et al*., 2013).

5.1.5.2 Antioxidant Metabolites

Antioxidant metabolites or non-enzymatic antioxidants include GSH, arginine, citrulline, taurine, creatine, selenium, zinc, vitamin E, C, A and tea polyphenols (Fang *et al*., 2002). The section below discusses the role of GSH as it plays a role in the one carbon cycle.

Glutathione

GSH was discovered in yeast cells in 1888 (Aoyama and Nakaki, 2013). It is the main non-protein thiol in cells whose functions are dependent on the redox-active thiol of its cysteine moiety that serves as a cofactor for a number of antioxidant and detoxifying enzymes (Ribas *et al*., 2014). GSH is a small redox-active molecule existing in two main stable forms: the thiol and the disulphide. Challenging environmental conditions are known to alter this ratio, notably by inducing the accumulation of GSSG (Rahantaniaina *et al*., 2013). GSH is responsible for supplying reducing equivalents to neutralize hydrogen peroxide, a toxic oxidizing agent that is produced during normal metabolism. Without GSH, hydrogen peroxide can rise to toxic levels in tissues and blood where it can cause severe oxidative injury to organs and to the microvasculature (Pravda, 2014). It is involved in nutrient metabolism, antioxidant defence and regulation of cellular metabolic functions ranging from gene

expression, DNA and protein synthesis to signal transduction, cell proliferation and apoptosis (Aquilano *et al*., 2014). GSH is the major antioxidant in the brain, and as such plays a key role in the detoxification of reactive oxidants (Carvalho *et al*., 2014; Aoyama and Nakaki, 2013). Several antioxidant systems depend on GSH (Mailloux *et al*., 2013).

5.1.6 Factors Affecting Oxidative Stress in Pregnancy

Pregnancy, placental abnormality and preexisting maternal constitutional conditions are the major factors influencing placental oxidative stress (Yang *et al.*, 2012). Maternal factors like age, smoking, socioeconomic status (SES), infections, and nutritional status influence oxidative stress (Langie *et al.*, 2012). Modern lifestyle including exposure to pollutants, poor diet and lack of exercise cause excess inflammation, oxidative stress and ultimately DNA damage which increases the risk of infertility, miscarriage and pregnancy complications (Furness *et al.*, 2011).

5.1.6.1 Maternal Nutrition

Maternal diet, specifically the intakes of antioxidants and polyunsaturated fats are implicated in the development of oxidative stress with subsequent effects on the offspring health (Thornburg *et al*., 2010; Turpeinen *et al.*, 1998).

Antioxidants

Antioxidant nutrients including vitamin E, vitamin C, vitamin B_6, beta-carotene, zinc and selenium have a beneficial role in oxidative stress (Hsu and Guo, 2002). The reduced levels of trace elements associated with inadequate amount of antioxidant enzymes are suggested to be important factors associated with oxidative

stress leading to endothelial dysfunction in preeclamptic mothers (Negi *et al.*, 2012). Further, antioxidants have a beneficial role in early status of brain development in rats against oxidative stress (Antonio-García and Massó-Gonzalez, 2008). Complementation with micronutrients during pregnancy is an early and innovative alternative to strengthen the prevention of chronic diseases in the population (Ramírez-Vélez *et al.*, 2011).

Oxidative stress resulting from folic acid deficiency is reported to be responsible for neural tube defects (Wegrzyn *et al*., 2013). Low folate supply during early life may leave an epigenetic mark that can predispose the offspring to further dietary insults, causing adverse effects during adult life (Langie *et al*., 2013). Supplementation with high-dose folic acid during pregnancy in mice is known to protect against lipopolysaccharide-induced neural tube defects through its anti-inflammatory and anti-oxidative effects (Zhao *et al*., 2014). Fortification of flour with folic acid has a major impact on neural tube defects in many countries (Castillo-Lancellotti *et al*., 2013).

Vitamin D supplementation for 9 weeks among pregnant women has been reported to have beneficial effects on oxidative stress (Asemi *et al.*, 2009). The neuroprotective effect of vitamin D is associated with its influence on neurotrophin production and release, neuromediator synthesis, intracellular calcium homeostasis, and prevention of oxidative damage to nervous tissue (Wrzosek *et al*., 2013).

Vitamins A, C, E are potent antioxidants (Poston *et al*., 2011) but their effect on oxidative stress markers has been inconsistent (Schnorr *et al*., 2011; Pasquali *et al*., 2010). Supplementation of these vitamins during pregnancy to reduce the risk of preeclampsia and preterm birth has not been consistent (Talaulikar and Manyonda, 2011; Bastani *et al*., 2011; Conde-Agudelo *et al*., 2011; Basaran *et al*., 2010; Pandey

et al., 2000). Studies suggest a need to investigate the optimum dose and timing of antioxidant administration (Woods *et al*., 2001; Buettner, 1993)

Selenium supplementation can up-regulate endogenous antioxidant systems and protect trophoblast cells from oxidative stress (Watson *et al*., 2012). Studies have reported that selenium could play an important role in adverse outcomes such as miscarriages, neural tube defects, diaphragmatic hernia, premature birth, low birth weight, preeclampsia, glucose intolerance and gestational diabetes (Mariath *et al*., 2011). Antioxidant activity of selenium is via its incorporation into selenoproteins such as glutathione peroxidase enzymes, levels of which have been shown to be reduced in miscarriage and preeclampsia (Mistry and Williams, 2011). It is important to maintain an adequate selenium supply in pregnancy complications (Khera *et al*., 2013).

Zinc deficiency during foetal life and lactation induces an early decrease in renal functional units, associated with a decrease in nitric oxide activity and an increase in oxidative stress (Tomat *et al*., 2013). Zinc is a widely studied microelement in infant feeding because it is a component of several enzymes involved in intermediary metabolism ranging from growth to cell differentiation and metabolism of proteins, carbohydrates, and lipids (Tomat *et al*., 2011).

Oxidative stress is a characteristic of copper (Cu) deficiency (Uriu-Adams *et al*., 2005). In dietary-induced foetal growth restriction, Cu deficiency plays a major etiologic role in the decrease of foetal growth and anti-oxidant capacity (Ergaz *et al*., 2012). Premature and low birth weight infants can be born with low Cu stores which may be due to increased oxidative stress (Uriu-Adams *et al*., 2010)

Iron, deficiency and excess, can result in free radical mitochondrial damage (Casanueva and Viteri, 2003). Supplementation with Fe and particularly in

combination with vitamins (riboflavin, folic acid) has been shown to improve the haematological status as well as oxidative stress in anaemic pregnant women (Ma *et al*., 2010).

Long Chain Polyunsaturated Fatty Acids

Cell membranes rich in LCPUFA are particularly susceptible to oxidative stress (Shichiri, 2014). Oxidative stress promotes damage to plasma membrane omega-3 fatty acids such as DHA thereby, disrupting neuronal signalling (Gomez-Pinilla and Tyagi, 2013). Primary prevention of cardiovascular disease and mental ill health starts, crucially, with maternal nutrition that includes consuming more omega-3 fatty acids to lower transient short-lived meal-induced oxidative stress (Robson, 2009).

Supplementation of omega-3 fatty acids have shown significantly reduced lipid peroxidation, nucleic acid and protein oxidation, thereby promoting neuronal and glial cell survival on brain tissue analysis of traumatic brain injury models (Kumar *et al*., 2014).

Omega-3 fatty acid supplementation is reported to have beneficial effects on oxidative stress supporting their role as antioxidants (Chaudhary *et al*., 2014; Fernández-Iglesias *et al*., 2013; Patten *et al*., 2013). Maternal dietary omega-3 fatty acid supplementation during pregnancy is reported to reduce placental oxidative damage and increase placental levels of pro-resolving mediators in rats (Jones *et al*., 2014). However, omega 3 fatty acids are also known to be highly susceptible to lipid peroxidation (Jones *et al*., 2013). During pregnancy the ability of DHA to alter placental pro-oxidant/antioxidant balance is dependent on the DHA concentration used and the gestational age of the placental tissue (Stark *et al*., 2013).

5.1.6.2 Maternal Characteristics influencing Oxidative Stress

Age

Increase in the risk of preeclampsia with maternal age could be due to an increase in oxidative stress associated with age (Hague *et al*., 2010, Tikhonova *et al*., 2014). Studies in animals have shown that delayed childbearing results in shortening of oviduct and somatic ovarian telomere length possibly due to increased oxidative stress (Aiken *et al*., 2013) and this may lead to several complications in pregnancy.

Weight

Inadequate or excessive pregnancy weight gain can complicate both maternal and foetal health (Agarwal *et al.*, 2012). Maternal obesity (BMI, 30-43 kg/m^2) is known to be associated with increased markers of inflammation and oxidative stress (Saben *et al*., 2014). Excess maternal weight gain during pregnancy elevates oxidative stress indicating a possible increased risk of CVD development in later life (Stefanović *et al*., 2012).

Increased oxidative stress is thought to be a very early response to poor maternal nutrition and suboptimal environment *in utero*, and these effects are exacerbated by accelerated postnatal growth (Tarry Adkins *et al*., 2013). Intrauterine malnutrition is associated with significant oxidative stress in small for gestational age neonates born at term to malnourished mothers (Gupta *et al*., 2004).

Socioeconomic Status

Lower SES is associated with higher oxidative stress and lower antioxidant levels (Janicki-Deverts *et al*., 2009). Poor people age faster than rich people due to the unhealthy environments and high oxidative stress to which they are exposed and

results in socioeconomic differences in health (Adams and White, 2004). The response of a person to resource scarcity depends on the harshness of their early-life environment and increase in oxidative stress (Griskevicius *et al*., 2013).

Exercise

A well-planned program of exercise is an important component of a healthy lifestyle and is also recommended during pregnancy (Golbidi and Laher, 2013). Exercise training can promote the synthesis and release of nitric oxide, improve angiogenesis and decrease oxidative stress (Gąsiorowski and Dutkiewicz, 2013). However excessive maternal activity during pregnancy is associated with smaller foetal size in rural India (Rao *et al*., 2003).

Alcohol/ Smoking/ Drug Addiction

The adverse effects of smoking and alcohol on birth outcomes have been well documented, and growing evidence indicates that exposure to either can delay the time to conception possibly through increases in oxidative stress (Ruder *et al*., 2009). Ethanol and nicotine, which harm the development of foetus, may induce both oxidative and endoplasmic reticulum stress responses in human placental trophoblastic cells (Repo *et al*., 2014). The ingestion of alcohol and/or cigarette smoke during pregnancy can lead to the generation of ROS and produce an imbalance in the intracellular redox state, leading to an overall increase in oxidative stress resulting in abnormal foetal development (Dittrich *et al*., 2012). Smoking mothers are reported to have more oxidative stress than non-smoking mothers (Aydogan *et al*., 2013). Environmental tobacco smoke in the early postnatal period adversely affects

pro-oxidative/antioxidative status within first weeks of life in very early infancy (Noakes *et al*., 2007).

5.1.7 Oxidative Stress and Antioxidants in Preeclampsia

Reports indicate that decreased levels of antioxidants and increased lipid peroxidation play an important role in the pathogenesis of preeclampsia (Genc *et al*., 2011; Karacay *et al*., 2010; Rani *et al*., 2010; Gupta *et al*., 2009; Patil *et al*., 2007; Rudra *et al*., 2006). Oxidative stress associated with preeclampsia may be a consequence of reduced antioxidant defence pathways. It has been suggested that GSH, GPx and antioxidant vitamin status contribute to the endothelial dysfunction and hypertension in preeclampsia (Mistry *et al*., 2008; Krishna Mohan and Venkataramana, 2007). Reduced levels of enzymatic antioxidants like SOD, catalase, GPx and glutathione reductase (GSR) have also been observed (Dordević *et al*., 2008; Vanderlie *et al*., 2008).

Several mechanisms have been proposed for the protection of developing embryo from ROS. An internal protection system exists which is comprised of both enzymatic and nonenzymatic antioxidant defences such as SOD, GPx and GSH (Finkel 2000). In addition there also exists an association between ROS and the enzyme eNOS which produces nitric oxide in endothelial cells which works as vasodilator and has been linked with preeclampsia (Kim *et al*., 2006).

Studies from our department and other studies have reported an imbalance between oxidative stress and antioxidants in women which is associated with poor birth outcome in preeclampsia (Hilali *et al*., 2013; Mehendale *et al*., 2008; Kulkarni *et al*., 2010). However, these results were observed when the pathology had progressed and may have been secondary to the effects of the disease. Further, it is suggested that

early pregnancy is susceptible to oxidative stress, and thus characterization of antioxidant systems would improve the understanding of placental development and function (Garrel *et al*., 2010).

Hypoxic conditions that prevail in the placenta after the first trimester increase the production of ROS and leads to oxidative stress associated with preeclampsia (Murray, 2012; Tal, 2012; van Patot *et al*., 2012). Oxidative damage leads to breaks in DNA providing access to sites for DNA methyltransferases, which promote DNA methylation (O'Hagan *et al*., 2011). Studies in humans and animal models have reported that oxidative stress plays an important pathophysiological role in the foetal programming of neurodevelopmental and metabolic diseases (Sartori *et al*., 2012, Estringer *et al*., 2012). A compromised pregnancy such as preeclampsia may expose the foetus to increased oxidative stress and through epigenetic changes may alter its development in later life (Chen *et al*., 2013).

Several longitudinal studies have examined changes in oxidative stress parameters across gestation in women with preeclampsia. However, these are on a small sample size (Genc *et al*., 2011; Loverro *et al*., 1996). They have mainly examined parameters like thiols, uric acid, thiobarbituric acid, vitamin E, vitamin C and selenium (Anastasakis *et al*., 2008; Roes *et al*., 2006). They are on a heterogeneous population and from varying ethnicities (Medrano Rodríguez *et al*., 2008; Ademuyiwa *et al*., 2007; Jahn *et al*., 1997) and had women that smoked (Rudra *et al*., 2006). These studies may be confounded by a heterogeneous population and smoking that may result in altered oxidative stress levels (Szanton *et al*., 2012; Noakes *et al*., 2007; Janicki-Deverts *et al*., 2009, Bizoń *et al*., 2011). Further, there are limited studies examining the association of maternal levels of oxidative stress

markers with those in the cord blood (Mihailović *et al*., 2000) or birth outcome (Min *et al*., 2009).

A departmental study in an animal model suggests that antioxidant defence system of the brain may be programmed during the critical developmental windows and may be the key mechanism leading to impaired cognition in later life (Roy *et al*., 2013). We hypothesize that an abnormal antioxidant defence mechanism exists early in pregnancy in mothers who develop preeclampsia. This may increase the risk for adverse foetal programming of metabolic and neurodevelopmental disorders in later life. Thus, there is a need to understand the levels of oxidative stress and antioxidant enzymes from early gestation until delivery in women with preeclampsia and examine their association with blood pressure (BP) and birth outcome.

This prospective study reports the maternal and cord MDA, SOD, GPx and GSH levels at different gestational time-points in women who developed preeclampsia as compared to normotensive controls (NC), associating it with the birth weight and maternal systolic and diastolic BP.

5.2 Materials and Methods

5.2.1 Study Subjects

The recruitment of study population and the inclusion and exclusion criteria are as mentioned in Chapter 3 (Section 3.2.1). Neonatal growth measures were recorded as mentioned in Chapter 3 (Section 3.2.1).

5.2.2 Power of the Study

The power of the study based on our earlier cross sectional study on MDA levels in mothers with preeclampsia using Power and Sample Size Calculation

software (version 3.0.43) (Mehendale *et al*., 2008). For 3 controls per preeclampsia subject we would need to study 46 preeclampsia subjects and 138 NC subjects to be able to reject the null hypothesis. The incidence of preeclampsia in this population is about 8-10% of total pregnancies. In view of this, during the period of our sample collection 148 women for NC group were selected randomly among which 123 had complete data for all three gestational time-points with cord blood samples. In case of preeclampsia, a total of 63 women were recruited out of which 34 women had complete data at all gestational time-points including cord samples.

5.2.3 Sample Collection, Processing and Storage

Blood sample collection was as described in Chapter 2 (Section 2.2.2) while placental sample collection was as described in Chapter 3 (Section 3.2.2)

5.2.4 Biochemical Estimations

MDA levels were measured from maternal and cord plasma using the Oxis Research TM Bioxytech® (New York, USA) MDA-586TM Spectrophotometric Assay and are expressed as µM/mL. Levels of GPx were measured from maternal and cord erythrocytes using Oxis Research TM Bioxytech® (New York, U.S.) GPx-340TM Colorimetric Assay for Cellular Glutathione Peroxidase and are expressed as mU/ml. SOD levels were measured from maternal and cord erythrocytes using Cayman's Superoxide Dismutase Assay Kit (Michigan, USA). SOD activity was expressed as U/ml. GSH levels were measured in maternal and cord erythrocytes using Cayman's (Michigan, USA) Glutathione Assay Kit and was expressed as µM/mL. eNOS levels were measured in placental homogenates using Quantikine Human eNOS Quantikine ELISA Kit by R&D Systems and was expressed as pg/ml.

5.2.5 Extraction of Total RNA, cDNA Synthesis and Quantitative Real Time (RT)- PCR Assays

Isolation of total RNA, cDNA synthesis and mRNA levels of *SOD, GPx, GAPDH* genes from NC and preeclampsia placental samples was performed. Total RNA from placenta samples was isolated using Trizol method and quantified by the Eppendorf Biophotometer Plus (Hamburg, Germany) by the method described by us earlier (Meher *et al*., 2014). For each placental sample, 2 μg of RNA was reverse transcribed to cDNA using the High-Capacity cDNA reverse transcription Kit from Applied Biosystems (California, USA). RT-PCR for *SOD, GPx* and *GAPDH* were erformed with the TaqMan Universal PCR Master Mix (California, USA) using 7500 Standard Real Time PCR system from Applied Biosystems (California, USA). The relative expression level of the gene of interest was examined with respect to *GAPDH*. Relative expression levels of genes were calculated and expressed as $2^{\Delta Ct}$. The following TaqMan® primers were used in this study: *GAPDH* (Hs99999905_m1); *SOD* (Hs00829989_gH); *GPx* (Hs00166575_m1).

5.2.6 Statistical Analysis

The data were analyzed using the SPSS/PC+ package (Version 20.0, Chicago, IL, USA). Continuous variables are reported as mean ± S.D and discrete variables as percent (%). Log_{10} transformation was used for skewed distributions. Mean values of the estimates of various parameters were compared at conventional levels of significance ($p<0.05$ and $p<0.01$) using the independent 't' test and one-way analysis of variance (ANOVA). The data was also analyzed after adjusting for gestational age and SES. Association between different parameters with birth outcome and BP were analyzed after adjusting for maternal age, BMI, gestational age and SES. In the

current study, the variable sample numbers for different time-points and different parameters are due to either loss in the follow up of subjects at various time-points or insufficient sample volume available. This study reports results before and after adjusting for confounders such as SES and gestation suggesting the importance of confounders and appropriate statistical tools while analyzing the data. However, most of the studies reported in literature have not defined confounders while comparing means of the parameters.

5.3 Results

5.3.1 Maternal and Neonatal Characteristics

The maternal body mass index (BMI) at all gestational time-points in women with preeclampsia was higher ($p<0.01$ for all) than NC women. Systolic BP of women with preeclampsia were higher ($p<0.01$ for all) at all time-points than NC women. Diastolic BP were higher at T2 and T3 ($p<0.01$ for both) in women with preeclampsia than NC women. The percentage of nulliparous women was higher in the preeclampsia group compared to the NC group (70% vs. 45%, $p<0.01$) (Table 7).

Table 7: Maternal and Neonatal Characteristics

	NC (n=148)	**PE (n=63)**
Maternal Characteristics		
Age in years	26 ± 4	26 ± 8
BMI in kg/m^2		
T1	22 ± 3	25 ± 5**
T2	24 ± 4	28 ± 6**
T3	25 ± 4	30 ± 6**
Gestation in weeks (range)		
T1	19 ± 2	18 ± 2**
T2	29 ± 3	30 ± 2
T3	39 ± 1	37 ± 3**
Sys BP in mmHg		
T1	112 ± 8	117 ± 12**
T2	113 ± 8	122 ± 13**
T3	120 ± 9	142 ± 18**
Dias BP in mmHg		
T1	73 ± 7	74 ± 8
T2	72 ± 7	76 ± 9**
T3	78 ± 6	94 ± 12**
Education in n (%)		
lower	35	22
higher	12	14
graduation	36	43
post graduation	18	21
Parity in n (%)		
nulliparous	45	70
multiparous	55	30
Income in INR (range)	26 ± 4	26 ± 8
Neonatal Characteristics		
Birth weight (Kg)	2.9 ± 0.3	2.8 ± 0.6**
Baby length (cm)	48.2 ± 2.8	47.4 ± 2.3
Baby head circumference (cm)	33.7 ± 1.3	33.2 ± 1.3*
Baby chest circumference (cm)	32.1 ± 1.6	31.5 ± 2.5*

Values are expressed as Mean ± S.D.; *p<0.05, **p<0.01 as compared to NC; **NC –** normotensive control; **PE –** preeclampsia;

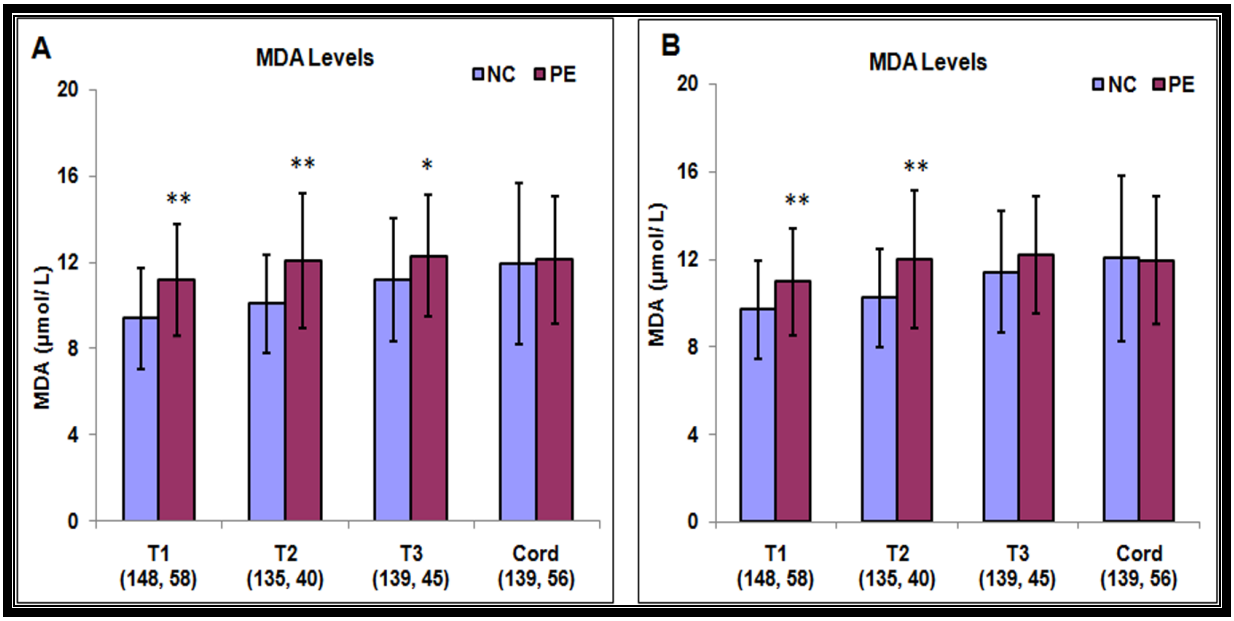
Neonatal characteristics show that birth weight ($p<0.01$), baby head and chest circumference ($p<0.05$) were lower in preeclampsia group as compared to the NC group (Table 7).

5.3.2 Maternal and Cord Malondialdehyde Levels

Maternal plasma MDA levels at T1 ($p<0.01$), T2 ($p<0.01$) and T3 ($p<0.05$) were higher in women with preeclampsia than those in NC women. No difference in cord plasma MDA levels were observed in preeclampsia group as compared to NC group. Levels of MDA were lower at only T1 ($p<0.01$) and T2 ($p<0.01$) after adjusting for gestational age and SES (Fig. 28 A & B).

In the NC group the MDA levels at T3 were significantly higher ($p<0.01$ for both) than T1 and T2. Similarly cord levels were also higher ($p<0.01$ for both) than T1 and T2. However in women with preeclampsia the levels of MDA did not vary across gestation.

Fig. 28: Levels of Maternal and Cord Plasma MDA at Different Time Points in Normotensive Control Women and Women with Preeclampsia



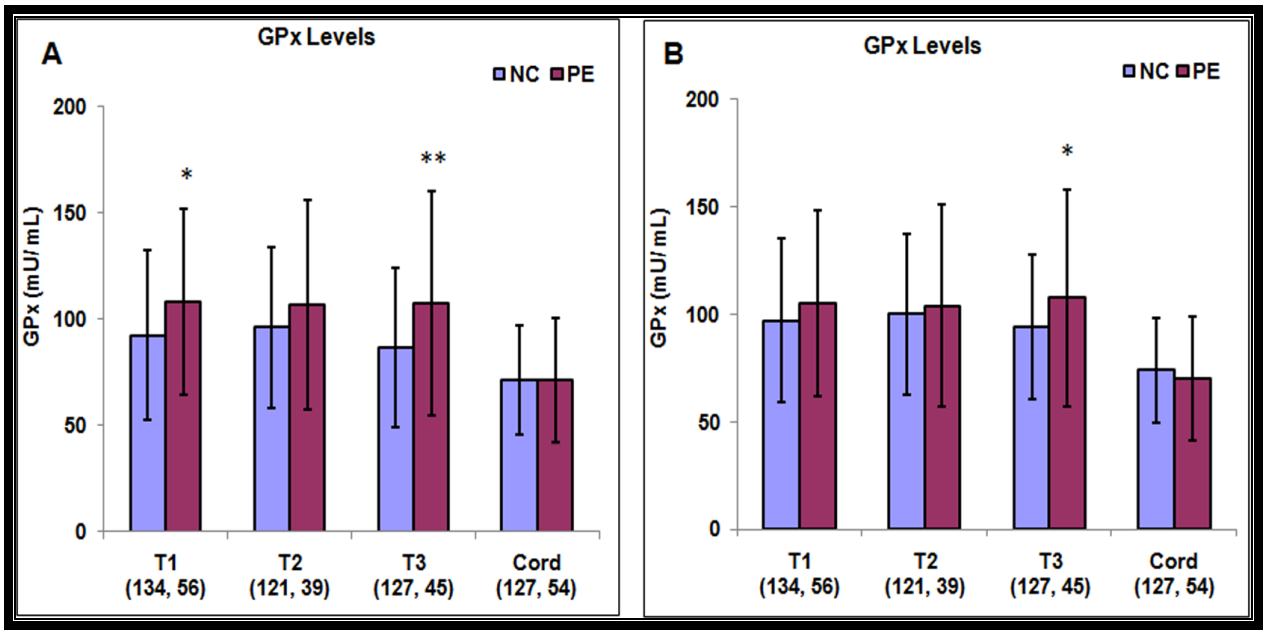
Values are expressed as Mean ± SD; *p<0.05, **p<0.01 as compared to NC. **A –** before adjusting for confounders; **B –** after adjusting for confounders; **MDA** - malondialdehyde; **NC** - normotensive control; **PE** - preeclampsia; **T1** - 16 to 20 weeks of gestation; **T2** - 26 to 30 weeks of gestation; **T3** - at delivery.

5.3.3 Maternal and Cord Glutathione Peroxidase Levels

There was higher maternal erythrocyte GPx levels at T1 (p<0.05) and T3 (p<0.01) in women with preeclampsia than NC (Fig. 29 A). After adjustment for gestational age and SES there were higher levels of GPx at only T3 (p<0.05) in women with preeclampsia than NC women (Fig. 29 B).

In the NC group the GPx levels were significantly lower (p<0.01) at T3 than T2. Cord GPx levels were lowest (p<0.01 for all) as compared to all the three i.e. T1, T2 and T3 time-points. However in women with preeclampsia the levels of GPx did not show any difference across gestation but was lowest (p<0.01 for all) in the cord as compared to all the time-points.

Fig. 29: Levels of Maternal and Cord Erythrocyte GPx at Different Time Points in Normotensive Women and Women with Preeclampsia



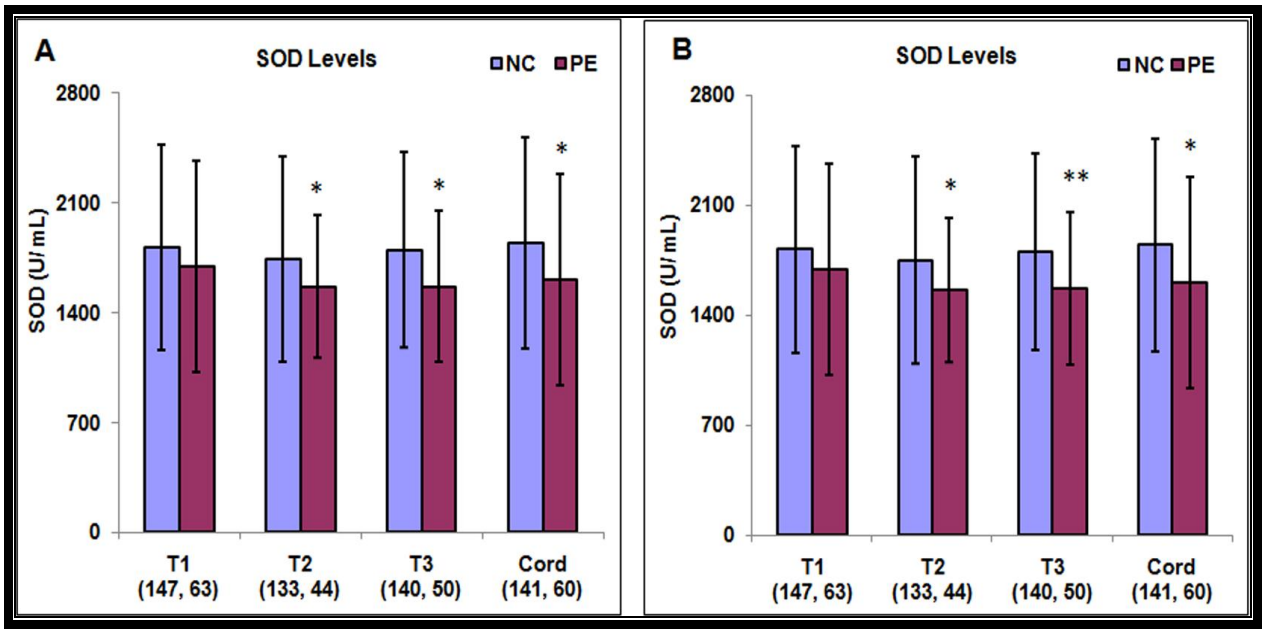
Values are expressed as Mean ± SD; *p<0.05, **p<0.01 as compared to NC; **A** – before adjusting for confounders; **B** – after adjusting for confounders; **GPx** – glutathione peroxidase; **NC** - normotensive control; **PE** - preeclampsia; **T1** - 16 to 20 weeks of gestation; **T2** - 26 to 30 weeks of gestation; **T3** - at delivery

5.3.4 Maternal and Cord Superoxide Dismutase Levels

The levels of maternal erythrocyte SOD were lower at T2 (p<0.05), T3 (p<0.05) and in cord (p<0.05) in the preeclampsia group than NC group (Fig. 30 A). After adjusting for gestational age and SES, SOD levels were lower at T2 (p<0.05), T3 (p<0.01) and in cord (p<0.05) in preeclampsia group than NC group (Fig. 30 B).

There was no longitudinal difference within the NC and preeclampsia groups.

Fig. 30: Levels of Maternal and Cord Erythrocyte SOD at Different Time Points in Normotensive Control Women and Women with Preeclampsia



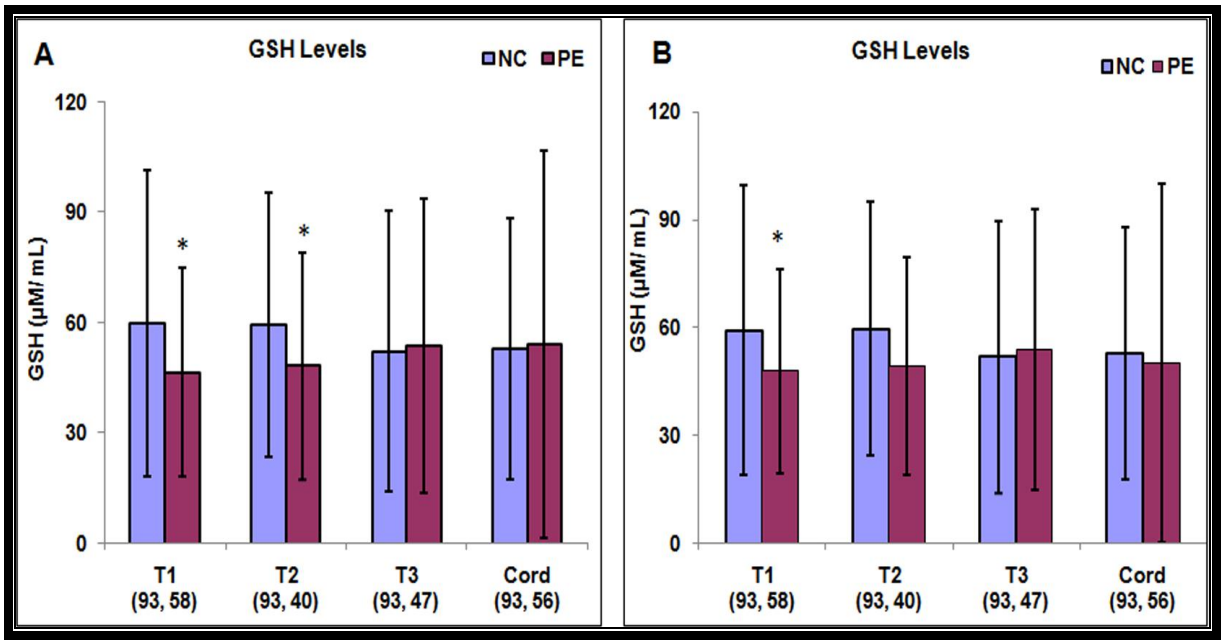
Values are expressed as Mean ± S.D.; *p<0.05, **p<0.01 as compared to NC. **A –** before adjusting for confounders; **B –** after adjusting for confounders; **NC -** normotensive control; **PE -** preeclampsia; **SOD –** superoxide dismutase; **T1 -** 16 to 20 weeks of gestation; **T2 -** 26 to 30 weeks of gestation; **T3 -** at delivery

5.3.5 Maternal and Cord Glutathione Levels

The maternal erythrocyte GSH levels at T1 ($p<0.05$) and T2 ($p<0.05$) were lower in women with preeclampsia than NC women (Fig. 31 A). However, after adjusting for gestational age and SES the GSH levels were lower only at T1 ($p<0.05$) in women with preeclampsia than NC (Fig. 31 B).

In the NC group the maternal GSH levels showed reducing trend towards term where the levels at T3 was lower ($p=0.05$) than T2 although it was not statistically significant. However in women with preeclampsia the levels of GSH did not show any difference across gestation.

Fig. 31: Levels of Maternal and Cord Erythrocyte GSH at Different Time-Points in Normotensive Control Women and Women with Preeclampsia

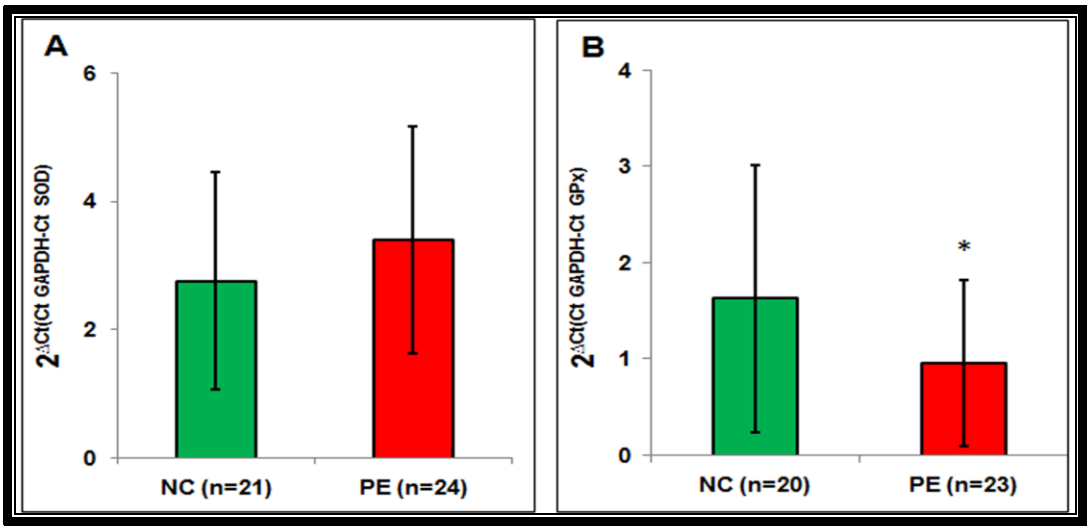


Values are expressed as Mean ± S.D.; *p<0.05. **A –** before adjusting for confounders; **B –** after adjusting for confounders; **GSH -** glutathione; **NC -** normotensive control; **PE -** preeclampsia; **T1 -** 16 to 20 weeks of gestation; **T2 -** 26 to 30 weeks of gestation; **T3 -** at delivery

5.3.6 Placental *SOD* and *GPx* Gene Expression

Placental *GPx* mRNA levels were lower (p<0.05) in women with preeclampsia than in NC women (Fig. 32 A). However placental *SOD* gene mRNA levels were comparatively higher in preeclampsia than NC group although it was not statistically significant (Fig. 32 B).

Fig. 32: Placental *SOD* and *GPx* Gene Expression

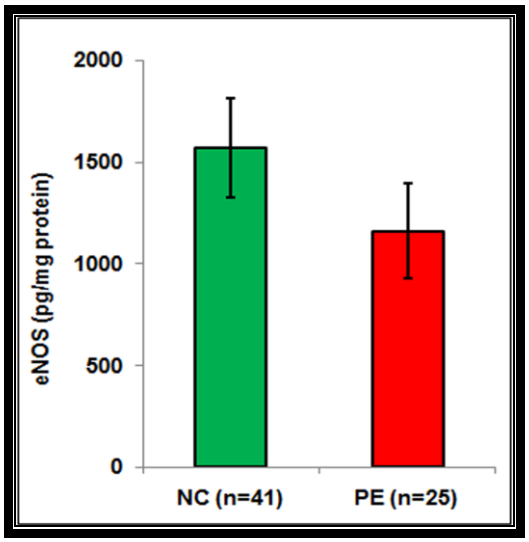


Values are expressed as Mean ± S.E.; *p<0.05 as compared to NC. **A –** expression of SOD; **B –** expression of GPx; **GPx –** glutathione peroxidase; **NC -** normotensive control; **PE -** preeclampsia; **SOD –** superoxide dismutase

5.3.7 Placental Endothelial Nitric Oxide Synthase Levels

Placental levels of eNOS were comparatively lower in preeclampsia than NC group although it was not statistically significant (Fig. 33).

Fig. 33: Placental Levels of eNOS



Values are expressed as Mean ± S.D.; **eNOS –** endothelial nitric oxide synthase **NC** - normotensive control; **PE** - preeclampsia;

5.3.8 Association of MDA with Blood Pressure

Maternal MDA was positively (r = 0.176, p = 0.049, n = 129) associated with diastolic BP at T2 in the NC group but not in the preeclampsia group.

5.3.9 Association of SOD with Blood Pressure

Maternal SOD was positively (r = 0.267, p = 0.043, n = 62) associated with diastolic BP at T1 in preeclampsia group.

5.3.10 Association of GPx with Blood Pressure and Birth Outcomes

Maternal GPx was negatively (r = -0.22, p = 0.02, n = 115) associated with diastolic BP at T2 in NC group. Maternal GPx of T3 was negatively (r = -0.195, p = 0.035, n = 122) associated with birth weight in NC group while at T2 it was positively (r = 0.386, p = 0.026, n = 37) associated with birth weight in preeclampsia group.

5.3.11 Association between Oxidative Stress Markers and Neurotrophins

Maternal MDA levels were negatively associated with BDNF at T2 (r = -0.198, p = 0.028, n = 124) in the whole cohort and at T2 (r = -0.368, p = 0.020, n = 40) in women with preeclampsia.

5.4 Discussion

This study demonstrates some important findings in the preeclampsia group (1) Higher plasma MDA levels at T1 and T2 (2) Higher erythrocyte GPx levels at T3 (3) Lower erythrocyte SOD at T2, T3 and in cord (3) Lower erythrocyte GSH levels at T1 (4) Lower expression of *GPx* in the placenta (5) The cord GPx was lowest as compared to maternal levels at all the three time-points (6) There was a positive

association of maternal SOD with diastolic BP at T1 and positive association of maternal GPx at T2 with birth weight. In addition in the NC group (1) MDA levels were highest at term while GPx levels were lowest in cord (2) Positive association between MDA and diastolic BP but a negative association between GPx and diastolic BP at T2. (3) Negative association between GPx of T3 with birth weight.

5.4.1 Higher MDA Levels in Women with Preeclampsia

In the current study, maternal plasma MDA levels were higher in women with preeclampsia at T1, and T2 as compared to NC women. However at delivery, the difference between preeclampsia and NC women MDA levels were significant only without adjusting for gestational age and SES. This is similar to studies which have shown higher MDA levels in serum, plasma and placental tissue samples in women with preeclampsia at delivery (Can *et al*., 2014; Siddiqui *et al*., 2013; Suhail *et al*., 2009). Further, higher MDA levels during 20–23 weeks of gestation have been reported in preeclampsia (Rudra *et al.* 2006, Anastasakis *et al*., 2008). Lipid peroxidation has been shown to induce endothelial dysfunction in experimental models, endothelial cell swelling, and damage cell membranes directly and reduced endothelium-dependent vasorelaxation (Molyneux *et al*., 2002). It has been suggested that higher MDA levels parallel the severity of preeclampsia (Wiktor and Kankofer, 2001). Reports indicate that oxidative stress is a cause of apoptosis in feto-placental unit which predates abnormalities in the foetus and foetal growth restriction (Nakatsukasa *et al*., 2013).

In the NC group MDA levels were highest at term and in cord blood. MDA level in umbilical cord blood is suggested to serve as an indication of perinatal oxidative stress (Gulbayzar *et al*., 2011). However, in women with preeclampsia the

levels of MDA were high right from T1 and remained similar throughout suggesting that this may be affecting the early developmental processes. MDA levels at T2 were positively associated with diastolic BP in a normotensive pregnancy and support studies which indicate that oxidative stress increases mid gestation possibly elevating blood pressure in normal pregnancy (Casanueva and Viteri, 2003).

5.4.2 Higher GPx Levels in Women with Preeclampsia

In this study maternal erythrocyte GPx levels were higher at delivery in women with preeclampsia as compared to NC women. There are several studies which report higher GPx levels in mothers with preeclampsia at term (Krishna Mohan and Venkataramana, 2007; Sharma *et al*., 2006; Llurba *et al*., 2004). In contrast lower maternal serum levels of GPx have also been reported (Negi *et al*., 2012).

In the NC group, the GPx levels were lower at term and lowest in cord. Similar trends were also observed in women with preeclampsia. This supports other studies which report lower levels of antioxidants like GPx, SOD and GSH at term (Patil *et al*., 2007; Davidge *et al*., 1992). In this study, the levels of GPx at T3 were negatively associated with birth weight in normal pregnancy while on contrary at delivery GPx levels were positively associated with birth weight in preeclampsia. Further, higher GPx levels were associated with reduced diastolic BP in normal pregnancy. Both the levels of GPx and MDA during T2 time-point and their associations with blood pressure indicate that the level of oxidative stress during this period of pregnancy affects endothelial functioning of the mothers. However further studies are required to understand these associations.

5.4.3 Lower SOD Levels in Women with Preeclampsia

In this study, lower levels of maternal erythrocyte SOD at T2, at delivery and in cord was observed in preeclampsia as compared to control. It has been reported that SOD levels are lower in women who develop preeclampsia at 10–14 and 20–24 weeks of gestation (Genc *et al*., 2011). Reports from cross-sectional studies indicate lower maternal and cord SOD levels in women with preeclampsia (Negi *et al*., 2012; Patil *et al*., 2009).

A positive association between maternal SOD and diastolic BP at T1 was observed in the preeclampsia group. SOD is responsible for production of H_2O_2 which if not converted to H_2O results in production of harmful radicals. It is reported that accumulation of H_2O_2 in kidney results in sustained hypertension and arterial BP (Makino *et al*., 2003). However, treatment with SOD mimetics has been reported to reduce blood pressure in rat models (Rodriguez-Iturbe *et al*., 2003).

5.4.4 Lower GSH Levels in Women with Preeclampsia

Maternal GSH levels were lower in women who develop preeclampsia as compared to NC women at T1. Reports indicate that GSH and GSH-related enzymes are involved in the metabolism and detoxification of cytotoxic compounds (Knapen *et al*., 1999). However, no change in placental or serum GSH levels in women with preeclampsia has also been reported (Das *et al*., 2012). A study in animals reports that reduced GSH causes significant increase in blood pressure (Vaziri *et al*., 2000).

In the present study, higher levels of GSH were observed during early pregnancy only in NC but not in preeclampsia women. Early embryos are sensitive to oxidative stress and mitochondria are known to provide ATP for GSH production to participate in the regeneration of NADPH (Dumollard *et al*., 2009).

5.4.5 Lower Placental Expression of *GPx* Gene in Women with Preeclampsia

In this study, there was a lower expression of *GPx* gene in the placenta in the preeclampsia group. This is in accordance with other studies that show highly significant reductions in expression of *GPx* in placentae from women with preeclampsia (Roland-Zejly *et al*., 2011; Mistry *et al*., 2010). The placenta is known to synthesize extracellular GPx (Avissar *et al*., 1994). Reduced expression of *GPx* and increased levels of GPx in maternal blood observed in this study may be due to the placental pathology of preeclampsia.

5.4.6 Lower Placental eNOS Levels in Women with Preeclampsia

In our study, the levels of eNOS were comparatively lower in preeclampsia than NC group but it was not statistically significant possibly due to small sample size. The altered production of nitric oxide may influence the reduced placental blood flow associated with preeclampsia. Our findings support earlier studies which report lower placental tissue of nitric oxide synthase in preeclampsia (Brennecke *et al*., 1997; Morris *et al*., 1995).

5.4.7 Negative Association between MDA and BDNF in Women with Preeclampsia

In this study maternal MDA levels were negatively associated with BDNF at T2 in the whole cohort and in women with preeclampsia. Oxidative stress is a factor that has been identified as important in the pathogenesis of neurological diseases (Crane, 2014). Antioxidant enzymes are associated with, and in some cases, prevent neuronal death in animal models of neurodegenerative diseases (Fujita *et al*., 2012).

Oxidants can traverse cell membranes to increase ROS and lipid peroxidation, activate different proteins and lead to DNA damage (Lewén *et al*., 2000). It has been shown that hydrogen peroxide treatment is capable of inducing apoptosis in nerve cells, which was released through the mitochondrial pathway under oxidative stress (Luo *et al*., 2010).

Growth factor signalling has the ability to control oxidative phosphorylation (Patel *et al*., 2013). The dual action of BDNF in neuronal metabolism and synaptic plasticity is crucial for activating signalling cascades under the action of diet and other environmental factors, using mechanisms of epigenetic regulation (Gomez-Pinilla and Tyagi, 2013; Jain *et al*., 2013).

This study demonstrates increased oxidative stress right from early pregnancy in women who develop preeclampsia. Studies from our department have earlier demonstrated and discussed that an altered one carbon cycle leads to increased homocysteine levels, thereby increasing the oxidative stress (Kulkarni *et al.* 2011). The inability of antioxidant enzymes to cope with increasing oxidative stress levels in women with preeclampsia is observed in this study.

This study also shows a negative association between oxidative stress and BDNF early in pregnancy in women who develop preeclampsia. Oxidative stress may cause epigenetic changes of antioxidant enzymes and neurotrophins in pregnant women increasing their risk to develop preeclampsia. It is likely that there may be epigenetic changes occurring at the gene specific level for antioxidant enzymes which can be studied further. Therefore, studies in our laboratory are currently focusing on the epigenetic mechanisms to explain the

increased oxidative stress in women with preeclampsia. These changes can link maternal nutrition, oxidative stress, altered placental development and foetal programming of adult NCDs.

SUMMARY

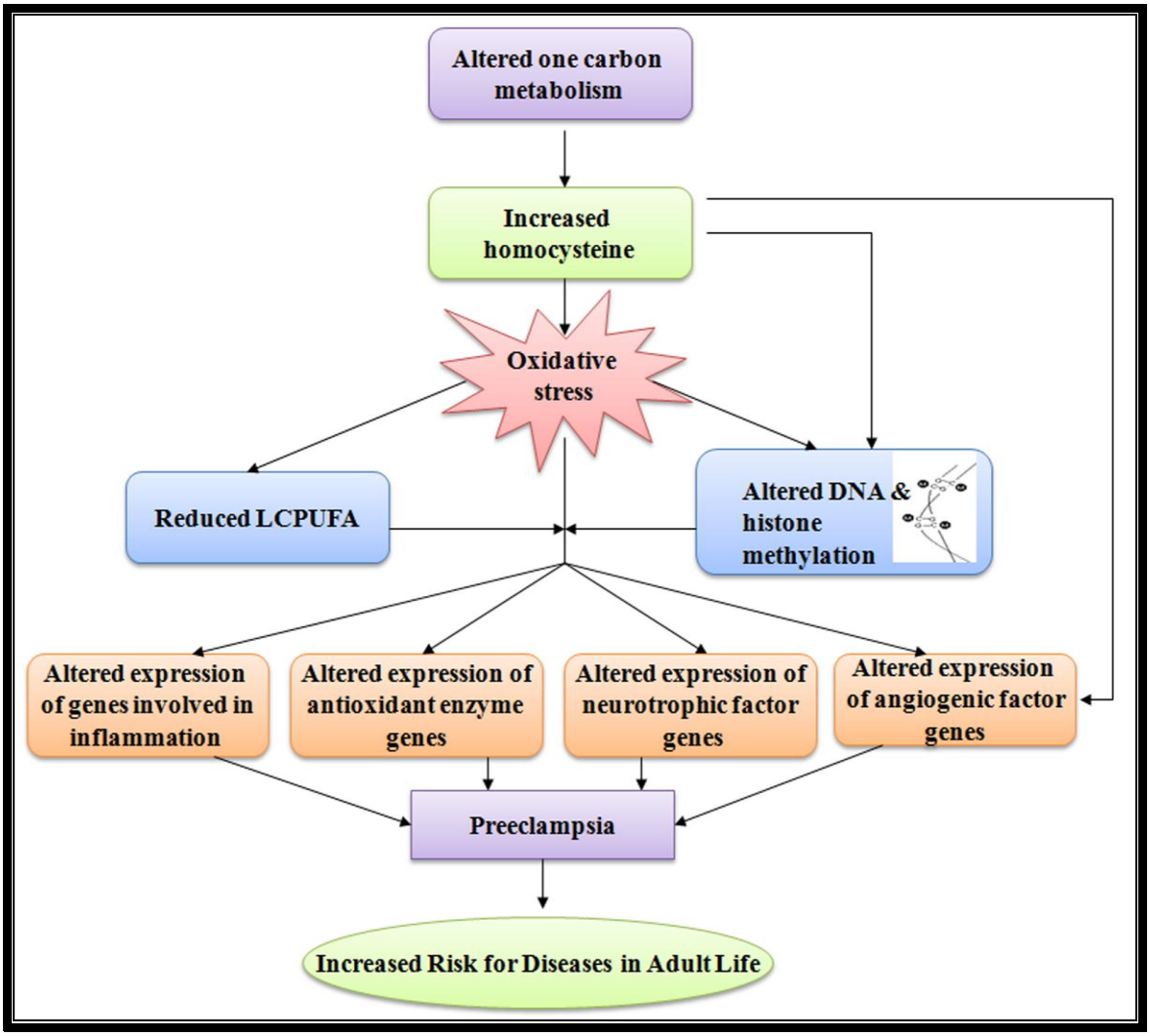
The present thesis entitled "Neurotrophins and Oxidative Stress in Preeclampsia" examines the levels of neurotrophins and oxidative stress markers in a cross sectional study on women recruited at delivery as well as in a prospective study at various time points across gestation. The cross sectional study was carried out on 95 normotensive control women and 106 women with preeclampsia (those delivering at term [n = 60] and those delivering preterm [n = 46]). The prospective study followed pregnant women at three time points i.e. 16 - 20 weeks of gestation (T1), at 26 - 30 weeks of gestation (T2) and the third at delivery (T3) and included 148 normotensive women women and 63 women with preeclampsia. The hypothesis of the study is "Oxidative stress, through the dysregulation of the one carbon cycle leads to altered levels and expression of antioxidant enzymes and neurotrophins in preeclampsia."

Preeclampsia is a pregnancy related disorder characterized by hypertension and proteinuria that develops after 20 weeks of gestation and the precise origins are unknown. Maternal nutrition is an important factor that contributes to adverse pregnancy outcomes such as preeclampsia. Studies carried out earlier in our department have shown altered levels of micronutrients (folic acid and vitamin B_{12}), lower docosahexaenoic acid (DHA), increased homocysteine and altered angiogenic balance at the end of pregnancy in women with preeclampsia. However, these results were observed when the pathology had progressed and may have been secondary to the effects of the disease.

Aberrations in the one carbon cycle leading to increased homocysteine have been suggested to induce oxidative stress. Oxidative stress is defined as an imbalance between the generation of reactive oxygen species and the antioxidants that prevent oxidative damages. Antioxidants like superoxide dismutase (SOD), catalase and

glutathione peroxidase (GPx) are known to play a key role in combating oxidative stress. Studies have shown that oxidative stress regulates neurotrophins such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF). These neurotrophins play an important role in the implantation, placental development and foetal growth. It is likely that an imbalanced one carbon cycle epigenetically influences the expression of various growth factors like neurotrophins and antioxidant enzymes. This will lead to pregnancy complications like preeclampsia and increase risk for diseases in children at later life. (Fig. 34)

Fig. 34: Altered One Carbon Metabolism Leads to Epigenetic Modification of Vital Genes (Antioxidant Enzymes and Neurotrophins) Resulting in Preeclampsia



DNA – deoxyribonucleic acid; **LCPUFA -** long chain polyunsaturated fatty acids

This is of significance since it is known that children born to mothers with preeclampsia are said to be at increased risk for developing behavioral problems during childhood and schizophrenia in later life.

The current study examines the levels of oxidative stress markers and neurotrophins from early pregnancy in women who develop preeclampsia. Further, the association of these parameters with blood pressure (BP) and birth outcome are also examined.

The Novel Findings from the Cross Sectional Study

This study for the first time demonstrates the following interesting findings

1. Women with preeclampsia (both delivering at term and preterm) had lower BDNF levels as compared to NC women.
2. Cord BDNF levels were higher in women with preeclampsia (term and preterm) as compared to the normotensive group.
3. Among the preeclampsia groups it was lower in the preterm-PE group as compared to the term-PE group. This may be due to either a preferential transport for brain-sparing in babies born to women with preeclampsia or impaired BDNF receptor signalling. Reduced cord BDNF levels observed in the current study may have implications for neonates born to women with preeclampsia delivering preterm, since they may be at increased risk of neurodevelopmental disorders in later life.
4. Maternal BDNF levels showed a negative correlation with blood pressure.

Our data based on altered angiogenesis reported earlier from our department and the reduced levels of BDNF in preeclampsia in the present study highlights the need to understand possible mechanisms underlying the association between angiogenesis and neurotrophins like BDNF in preeclampsia.

The Novel Findings from the Prospective Study:

Women with preeclampsia demonstrated the following

1. Higher plasma malondialdehyde (MDA) levels at T1 and T2 as compared to NC women indicating presence of increased oxidative stress in women with preeclampsia from early pregnancy.
2. Higher erythrocyte GPx levels at T3 as compared to normotensive control women.
3. Lower erythrocyte SOD at T2, T3 and in cord as compared to NC women.
4. Lower erythrocyte GSH levels at T1 as compared to NC women.
5. Lower expression of GPx in the placenta of women with preeclampsia as compared to NC women. The placenta is known to synthesize extracellular glutathione peroxidase and reduced expression observed in this study may be due to the placental pathology of preeclampsia.
6. Lower maternal BDNF levels at T1 and T3 in women with preeclampsia than those in NC women may be partly responsible for abnormal foeto-placental development in preeclampsia.
7. Lower levels of cord BDNF was also observed in preeclampsia which may have long-lasting consequences in their babies.

8. The lower placental BDNF gene expression levels observed in women with preeclampsia may be associated with impaired placental development in preeclampsia.
9. Maternal NGF levels in women with preeclampsia were comparable to NC women at all time points.
10. Cord NGF levels at delivery were higher in women with preeclampsia as compared to NC women
11. A negative association was observed between maternal diastolic BP and NGF levels at T1 in women with preeclampsia.
12. There was a positive association between cord NGF levels and baby head and chest circumference in the whole cohort.

The current study demonstrates increased oxidative stress from early pregnancy in women who develop preeclampsia. This study also shows the inability of antioxidant enzymes to cope with increasing oxidative stress levels in women with preeclampsia. Lower maternal BDNF levels in women with preeclampsia during early pregnancy may be implicated in the improper placental development. This study also shows a negative association between oxidative stress and BDNF early in pregnancy in women who develop preeclampsia. It is likely that oxidative stress may be associated with epigenetic changes of antioxidant enzymes and neurotrophins in preeclampsia.

IMPLICATIONS OF THE STUDY

This study for the first time examines the levels of oxidative stress markers and neurotrophins from early pregnancy in women who develop preeclampsia. The

current study provides a plausible molecular mechanism leading to preeclampsia. Our findings suggest that altered levels of neurotrophins and oxidative stress may affect the placental development in preeclampsia. Lower levels of BDNF in the cord may have implications for altered neurodevelopment in children born with preeclampsia.

SOCIETAL RELEVANCE OF THE STUDY

The incidence of preeclampsia is increasing in developing countries like India making it an important health issue. Therefore, there is an urgent need to understand the mechanisms of preeclampsia in order to develop strategies for prevention and treatment. The current study highlights the role of oxidative stress and neurotrophins in the pathophysiology of preeclampsia from early pregnancy.

FUTURE DIRECTIONS

Future studies need to be undertaken to examine gene specific methylation of the antioxidant enzymes and neurotrophin genes. This will help in understanding the epigenetic mechanism involved in the pathogenesis of preeclampsia. There is also a need to follow-up children born to mothers with preeclampsia to identify whether they are at risk of developing neurodevelopmental disorders in later life.

REFERENCES

Akcakus M, Altunay L, Yikilmaz A, Yazici C, Koklu E. The relationship between abdominal aortic intima-media thickness and lipid profile in neonates born to mothers with preeclampsia. J Pediatr Endocrinol Metab. 2010; 23:1143-9.

Alexander BT, Henry Dasinger J, Intapad S. Effect of Low Birth Weight on Women's Health. Clin Ther. 2014; 36:1913-23.

Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int J Biochem Cell Biol. 2010; 42:1634-50.

Al-Jameil N, Aziz Khan F, Fareed Khan M, Tabassum H. A brief overview of preeclampsia. J Clin Med Res. 2014; 6:1-7.

Aloe L, Rocco ML, Bianchi P, Manni L. Nerve growth factor: from the early discoveries to the potential clinical use. J Transl Med. 2012; 10:239.

Andero R, Ressler KJ. Fear extinction and BDNF: translating animal models of PTSD to the clinic. Genes Brain Behav. 2012; 11:503-12.

Andraweera PH, Dekker GA, Dissanayake VH, Bianco-Miotto T, Jayasekara RW, Roberts CT. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri-Lanka. J Matern Fetal Neonatal Med. 2013; 26:532-6.

Arévalo JC, Wu SH. Neurotrophin signaling: many exciting surprises! Cell Mol Life Sci. 2006; 63:1523-37.

Aukes AM, Wessel I, Dubois AM, Aarnoudse JG, Zeeman GG. Self-reported cognitive functioning in formerly eclamptic women. Am J Obstet Gynecol. 2007; 197: 365.e1-6.

Bae H, Ra Y, Han C, Kim DJ. Decreased serum level of NGF in alcohol-dependent patients with declined executive function. Neuropsychiatr Dis Treat. 2014; 10:2153-2157.

Bagnyukova TV, Powell CL, Pavliv O, Tryndyak VP, Pogribny IP. Induction of oxidative stress and DNA damage in rat brain by a folate/methyl-deficient diet. Brain Res. 2008; 1237:44-51.

Balaratnasingam S, Janca A. Brain Derived Neurotrophic Factor: a novel neurotrophin involved in psychiatric and neurological disorders. Pharmacol Ther. 2012; 134:116-24.

Balogun KA, Cheema SK. The expression of neurotrophins is differentially regulated by ω-3 polyunsaturated fatty acids at weaning and postweaning in C57BL/6 mice cerebral cortex. Neurochem Int. 2014; 66:33-42.

Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J. 1982; 1:549-53.

Barker DJ, Eriksson JG, Forsén T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002; 31:1235-9.

Barker DJ, Thornburg KL. Placental programming of chronic diseases, cancer and lifespan: a review. Placenta. 2013; 34:841-5.

Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. Environ Health. 2012; 11:42.

Basso O, Rasmussen S, Weinberg CR, Wilcox AJ, Irgens LM, Skjaerven R. Trends in fetal and infant survival following preeclampsia. JAMA. 2006; 296:1357-62.

Bath KG, Schilit A, Lee FS. Stress effects on BDNF expression: effects of age, sex, and form of stress. Neuroscience. 2013; 239:149-56.

Bazan NG, Calandria JM, Serhan CN. Rescue and repair during photoreceptor cell renewal mediated by docosahexaenoic acid-derived neuroprotectin D1. J Lipid Res. 2010; 51:2018-31.

Benn K, Passos M, Jayaram A, Harris M, Bongiovanni AM, Skupski D, Witkin SS. Association between neurotrophin 4 and long-chain polyunsaturated fatty acid levels in mid-trimester amniotic fluid. Reprod Sci. 2014; 21:1395-400.

Berti C, Cetin I, Agostoni C, Desoye G, Devlieger R, Emmett PM, Ensenauer R, Hauner H, Herrera E, Hoesli I, Krauss-Etschmann S, Olsen SF, Schaefer-Graf U, Schiessl B, Symonds ME, Koletzko B. Pregnancy and infants' outcome: nutritional and metabolic implications. Crit Rev Food Sci Nutr. 2014 (In press)

Bevilacqua E, Gomes SZ, Lorenzon AR, Hoshida MS, Amarante-Paffaro AM. NADPH oxidase as an important source of reactive oxygen species at the mouse maternal-fetal interface: putative biological roles. Reprod Biomed Online. 2012; 25:31-43.

Bhatia HS, Agrawal R, Sharma S, Huo YX, Ying Z, Gomez-Pinilla F. Omega-3 fatty acid deficiency during brain maturation reduces neuronal and behavioral plasticity in adulthood. PLoS One. 2011; 6:e28451.

Bibel M, Barde YA. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev. 2000; 14:2919-37.

Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors. 2004; 22:123-31.

Biondi C, Pavan B, Lunghi L, Fiorini S, Vesce F. The role and modulation of the oxidative balance in pregnancy. Curr Pharm Des. 2005; 11:2075-89.

Bisai S, Mahalanabis D, Sen A, Bose K, Datta N. Maternal early second trimester pregnancy weight in relation to birth outcome among Bengalee Hindus of Kolkata, India. Ann Hum Biol. 2007; 34:91-101.

Bischof P, Irminger-Finger I. The human cytotrophoblastic cell, a mononuclear chameleon. Int J Biochem Cell Biol. 2005; 37:1-16.

Bloomfield FH, Jaquiery AL, Oliver MH. Nutritional regulation of fetal growth. Nestle Nutr Inst Workshop Ser. 2013; 74:79-89.

Boehme F, Gil-Mohapel J, Cox A, Patten A, Giles E, Brocardo PS, Christie BR. Voluntary exercise induces adult hippocampal neurogenesis and BDNF expression in a rodent model of fetal alcohol spectrum disorders. Eur J Neurosci. 2011; 33:1799-811.

Bourre JM. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. J Nutr Health Aging. 2006; 10:377-85.

Bradshaw RA, Murray-Rust J, Ibáñez CF, McDonald NQ, Lapatto R, Blundell TL. Nerve growth factor: structure/function relationships. Protein Sci. 1994; 3:1901-13.

Branchi I, D'Andrea I, Fiore M, Di Fausto V, Aloe L, Alleva E. Early social enrichment shapes social behavior and nerve growth factor and brain-derived neurotrophic factorlevels in the adult mouse brain. Biol Psychiatry. 2006; 60:690-6.

Branchi I, Francia N, Alleva E. Epigenetic control of neurobehavioural plasticity: the role of neurotrophins. Behav Pharmacol. 2004; 15:353-62.

Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. Placenta. 2012; 33 S Int J Mol Sci. 2014; 15:16153-85.

Brown MC, Best KE, Pearce MS, Waugh J, Robson SC, Bell R. Cardiovascular disease risk in women with pre-eclampsia: systematic review and meta-analysis. Eur J Epidemiol. 2013; 28:1-19.

Brozović G, Sklebar I, Sunko BM, Blagaić V. Neurological disorders in pregnancy. Acta Med Croatica. 2012; 66:81-4.

Bulló M, Peeraully MR, Trayhurn P, Folch J, Salas-Salvadó J. Circulating nerve growth factor levels in relation to obesity and the metabolic syndrome in women. Eur J Endocrinol. 2007; 157:303-10.

Burdge GC, Lillycrop KA. Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease. Annu Rev Nutr. 2010; 30:315-39.

Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. Reproduction. 2009; 138:895-902

Burton GJ, Jauniaux E, Charnock-Jones DS. The influence of the intrauterine environment on human placental development. Int J Dev Biol. 2010; 54:303-12.

Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol. 2011; 25:287-99.

Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. J Clin Endocrinol Metab. 2002; 87:2954-9.

Bus BA, Molendijk ML, Penninx BJ, Buitelaar JK, Kenis G, Prickaerts J, Elzinga BM, Voshaar RC. Determinants of serum brain-derived neurotrophic factor. Psychoneuroendocrinology. 2011; 36:228-39.

Cabrera-Vásquez S, Navarro-Tableros V, Sánchez-Soto C, Gutiérrez-Ospina G, Hiriart M. Remodelling sympathetic innervation in rat pancreatic islets ontogeny. BMC Dev Biol. 2009; 9:34.

Campoy C, Escolano-Margarit MV, Anjos T, Szajewska H, Uauy R. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. Br J Nutr. 2012; 107:S85-106.

Cannon TD, Yolken R, Buka S, Torrey EF; Collaborative Study Group on the Perinatal Origins of Severe Psychiatric Disorders. Decreased neurotrophic response to birth hypoxia in the etiology of schizophrenia. Biol Psychiatry. 2008; 64:797-802.

Caporali A, Emanueli C. Cardiovascular actions of neurotrophins. Physiol Rev. 2009; 89:279-308.

Carlson BM. Patten's Foundations of Embryology, Ed 6, New York, McGraw-Hill, 1996, 180.

Carr DB, Epplein M, Johnson CO, Easterling TR, Critchlow CW. A sister's risk: family history as a predictor of preeclampsia. Am J Obstet Gynecol. 2005; 193:965-72.

Carter AM. Evolution of placental function in mammals: the molecular basis of gas and nutrient transfer, hormone secretion, and immune responses. Physiol Rev. 2012; 92:1543-76.

Carvalho AL, Caldeira MV, Santos SD, Duarte CB. Role of the brain-derived neurotrophic factor at glutamatergic synapses. Br J Pharmacol. 2008; 153 Suppl 1: S310-24.

Castro-Rendón WA, Castro-Alvarez JF, Guzmán-Martinez C, Bueno-Sanchez JC. Blastocyst-endometrium interaction: intertwining a cytokine network. Braz J Med Biol Res. 2006; 39:1373-85.

Cerdeira AS, Karumanchi SA. Angiogenic factors in preeclampsia and related disorders. Cold Spring Harb Perspect Med. 2012; 2: a006585.

Chaldakov GN, Fiore M, Stankulov IS, Manni L, Hristova MG, Antonelli A, Ghenev PI, Aloe L. Neurotrophin presence in human coronary atherosclerosis and metabolic syndrome: a role for NGF and BDNF incardiovascular disease? Prog Brain Res. 2004; 146:279-89.

Champagne FA. Epigenetic mechanisms and the transgenerational effects of maternal care. Front Neuroendocrinol. 2008; 29:386-97.

Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci. 2003; 4:299-309.

Chaves RN, Alves AM, Lima LF, Matos HM, Rodrigues AP, Figueiredo JR. Role of nerve growth factor (NGF) and its receptors in folliculogenesis. Zygote. 2013; 21:187-97.

Chen CP. Placental villous mesenchymal cells trigger trophoblast invasion. Cell Adh Migr. 2014; 8:94-7.

Chen DB, Zheng J. Regulation of placental angiogenesis. Microcirculation. 2014; 21:15-25.

Chen ZP, Zhou HL, Cao LS, Gao R. Significance of nerve growth factor expression in the prostate tissue of spontaneously hypertensive rats. Zhonghua Nan Ke Xue. 2011; 17:781-4.

Chouthai NS, Sampers J, Desai N, Smith GM. Changes in neurotrophin levels in umbilical cord blood from infants with different gestational ages and clinical conditions. Pediatr Res. 2003; 53:965-9.

Ciccolini F, Svendsen CN. Neurotrophin responsiveness is differentially regulated in neurons and precursors isolated from the developing striatum. J Mol Neurosci. 2001; 17:25-33.

Cindrova-Davies T. The therapeutic potential of antioxidants, ER chaperones, NO and H2S donors, and statins for treatment of preeclampsia. Front Pharmacol. 2014; 5:119.

Cirulli F, Alleva E. The NGF saga: from animal models of psychosocial stress to stress-related psychopathology. Front Neuroendocrinol. 2009; 30:379-95.

Comasco E, Sylvén SM, Papadopoulos FC, Oreland L, Sundström-Poromaa I, Skalkidou A. Postpartum depressive symptoms and the BDNF Val66Met functional polymorphism: effect of season of delivery. Arch Womens Ment Health. 2011; 14:453-63.

Conde-Agudelo A, Romero R, Kusanovic JP, Hassan SS. Supplementation with vitamins C and E during pregnancy for the prevention of preeclampsia and other adverse maternal and perinatal outcomes: a systematic review and metaanalysis. Am J Obstet Gynecol. 2011; 204:503.e1-12.

Craici I, Wagner S, Garovic VD. Preeclampsia and future cardiovascular risk: formal risk factor or failed stress test? Ther Adv Cardiovasc Dis. 2008; 2:249-59.

Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr. 2012; 3:21-38.

Cunha C, Brambilla R, Thomas KL. A simple role for BDNF in learning and memory? Front Mol Neurosci. 2010; 3:1.

Das UN. Folic acid and polyunsaturated fatty acids improve cognitive function and prevent depression, dementia, and Alzheimer's disease--but how and why? Prostaglandins Leukot Essent Fatty Acids. 2008; 78:11-9.

Dauncey MJ. Recent advances in nutrition, genes and brain health. Proc Nutr Soc. 2012; 71:581-91.

Davis EF, Newton L, Lewandowski AJ, Lazdam M, Kelly BA, Kyriakou T, Leeson P. Pre-eclampsia and offspring cardiovascular health: mechanistic insights from experimental studies. Clin Sci (Lond). 2012; 123:53-72.

Deinhardt K. and Jeanneteau F. Protein Phosphorylation in Human Health 2012; 7: 217-232.

Demir R, Yaba A, Huppertz B. Vasculogenesis and angiogenesis in the endometrium during menstrual cycle and implantation. Acta Histochem. 2010; 112:203-14.

Dennery PA. Oxidative stress in development: nature or nurture? Free Radic Biol Med. 2010; 49:1147-51.

Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. Molecular cues to implantation. Endocr Rev. 2004; 25:341–373.

Dhobale M, Joshi S. Altered maternal micronutrients (folic acid, vitamin B(12)) and omega 3 fatty acids through oxidative stress may reduce neurotrophic factors in preterm pregnancy. J Matern Fetal Neonatal Med. 2012; 25:317-23.

Dhobale M, Mehendale S, Pisal H, D'Souza V, Joshi S. Association of brain-derived neurotrophic factor and tyrosine kinase B receptor in pregnancy. Neuroscience. 2012a; 216:31-7.

Dhobale M, Mehendale S, Pisal H, Nimbargi V, Joshi S. Reduced maternal and cord nerve growth factor levels in preterm deliveries. Int J Dev Neurosci. 2012b; 30:99-103.

Dieni S, Rees S. BDNF and TrkB protein expression is altered in the fetal hippocampus but not cerebellum after chronic prenatal compromise. Exp Neurol. 2005; 192:265-73.

Dinçel N, Ünalp A, Kutlu A, Öztürk A, Uran N, Ulusoy S. Serum nerve growth factor levels in autistic children in Turkish population: a preliminary study. Indian J Med Res. 2013; 138:900-3.

Dixon CE, Flinn P, Bao J, Venya R, Hayes RL. Nerve growth factor attenuates cholinergic deficits following traumatic brain injury in rats. Exp Neurol. 1997; 146:479-90.

Do KQ, Cabungcal JH, Frank A, Steullet P, Cuenod M. Redox dysregulation, neurodevelopment, and schizophrenia. Curr Opin Neurobiol. 2009; 19:220-30.

Donkena KV, Young CY, Tindall DJ. Oxidative stress and DNA methylation in prostate cancer. Obstet Gynecol Int. 2010; 2010:302051.

Donovan MJ, Lin MI, Wiegn P, Ringstedt T, Kraemer R, Hahn R, Wang S, Ibañez CF, Rafii S, Hempstead BL. Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. Development. 2000; 127:4531-40.

Doridot L, Miralles F, Barbaux S, Vaiman D. Trophoblasts, invasion, and microRNA. Front Genet. 2013; 4:248.

D'Souza VA, Kilari AS, Joshi AA, Mehendale SS, Pisal HM, Joshi SR. Differential regulation of brain-derived neurotrophic factor in term and preterm preeclampsia. Reprod Sci. 2014; 21:230-5.

Duda DG, Jain RK. Pleiotropy of tissue-specific growth factors: from neurons to vessels via the bone marrow. J Clin Invest. 2005; 115:596-8.

Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry. 2006; 59:1116-27.

Duman RS, Voleti B. Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. Trends Neurosci. 2012; 35:47-56.

Dwivedi Y. Involvement of brain-derived neurotrophic factor in late-life depression. Am J Geriatr Psychiatry. 2013; 21:433-49.

Ehrenstein V, Rothman KJ, Pedersen L, Hatch EE, Sørensen HT. Pregnancy-associated hypertensive disorders and adult cognitive function among Danish conscripts. Am J Epidemiol. 2009; 170:1025-31.

Engeham SF1, Haase A, Langley-Evans SC. Supplementation of a maternal low-protein diet in rat pregnancy with folic acid ameliorates programming effectsupon feeding behaviour in the absence of disturbances to the methionine-homocysteine cycle. Br J Nutr. 2010; 103:996-1007.

Ernsberger U. Role of neurotrophin signalling in the differentiation of neurons from dorsal root ganglia and sympathetic ganglia. Cell Tissue Res. 2009; 336:349-84.

Ezzati M, Riboli E. Can noncommunicable diseases be prevented? Lessons from studies of populations and individuals. Science. 2012; 337:1482-7.

Faas MM, Spaans F, De Vos P. Monocytes and macrophages in pregnancy and pre-eclampsia. Front Immunol. 2014; 5:298.

Fall CH. Fetal programming and the risk of noncommunicable disease. Indian J Pediatr. 2013; 80:S13-20.

Fargali S, Sadahiro M, Jiang C, Frick AL, Indall T, Cogliani V, Welagen J, Lin WJ, Salton SR. Role of neurotrophins in the development and function of neural circuits that regulate energy homeostasis. J Mol Neurosci. 2012; 48:654-9.

Ferris LT1, Williams JS, Shen CL. The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. Med Sci Sports Exerc. 2007; 39:728-34.

Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000; 408:239-47.

Fiore M, Chaldakov GN, Aloe L. Nerve growth factor as a signaling molecule for nerve cells and also for the neuroendocrine-immune systems. Rev Neurosci. 2009; 20:133-45.

Forges T, Monnier-Barbarino P, Alberto JM, Guéant-Rodriguez RM, Daval JL, Guéant JL. Impact of folate and homocysteine metabolism on human reproductive health. Hum Reprod Update. 2007; 13:225-38.

Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation. 2006; 113:1708-14.

Friel S, Bowen K, Campbell-Lendrum D, Frumkin H, McMichael AJ, Rasanathan K. Climate change, noncommunicable diseases, and development: the relationships and common policy opportunities. Annu Rev Public Health. 2011; 32:133-47.

Fujita K, Tatsumi K, Kondoh E, Chigusa Y, Mogami H, Fujii T, Yura S, Kakui K, Konishi I. Differential expression and the anti-apoptotic effect of human placental neurotrophins and their receptors. Placenta. 2011; 32:737-44.

Fukuoka H, Mukai S, Taniguchi T. Nutritional environment in utero and development of obesity. Nihon Rinsho. 2013; 71:237-43.

Gacche RN, Meshram RJ. Targeting tumor micro-environment for design and development of novel anti-angiogenic agents arresting tumor growth. Prog Biophys Mol Biol. 2013; 113:333-54.

Gambling L, McArdle HJ. Iron, copper and fetal development. Proc Nutr Soc. 2004; 63:553-62.

Garcés MF, Sanchez E, Torres-Sierra AL, Ruíz-Parra AI, Angel-Müller E, Alzate JP, Sánchez ÁY, Gomez MA, Romero XC, Castañeda ZE, Sanchez-Rebordelo E, Diéguez C, Nogueiras R, Caminos JE. Brain-derived neurotrophic factor is expressed in rat and human placenta and its serum levels are similarly regulated throughout pregnancy in both species. Clin Endocrinol (Oxf). 2014; 81:141-51.

Giaginis C, Theocharis S, Tsantili-Kakoulidou A. Current toxicological aspects on drug and chemical transport and metabolism across the human placental barrier. Expert Opin Drug Metab Toxicol. 2012; 8:1263-75.

Gilbert JS, Ryan MJ, LaMarca BB, Sedeek M, Murphy SR, Granger JP. Pathophysiology of hypertension during preeclampsia: linking placental ischemia with endothelial dysfunction. Am J Physiol Heart Circ Physiol. 2008; 294:H541-50.

Gioiosa L, Iannitelli A, Aloe L. Stress, anxiety schizophrenia and neurotrophic factors: the pioneer studies with nerve growth factor. Riv Psichiatr. 2009; 44:88-94.

Gluckman PD, Hanson MA, Beedle AS. Early life events and their consequences for later disease: a life history and evolutionary perspective. Am J Hum Biol. 2007; 19:1-19.

Gomes EC, Silva AN, de Oliveira MR. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. Oxid Med Cell Longev. 2012; 2012:756132.

Gomes MV, Pelosi GG. Epigenetic vulnerability and the environmental influence on health. Exp Biol Med (Maywood). 2013; 238:859-65.

Gomez-Pinilla F, Tyagi E. Diet and cognition: interplay between cell metabolism and neuronal plasticity. Curr Opin Clin Nutr Metab Care. 2013; 16:726-33.

Gong JS, Kim GJ. The role of autophagy in the placenta as a regulator of cell death. Clin Exp Reprod Med. 2014; 41:97-107.

Gray JD, Milner TA, McEwen BS. Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. Neuroscience. 2013; 239:214-27.

Greabu M, Battino M, Mohora M, Olinescu R, Totan A, Didilescu A. Oxygen, a paradoxical element? Rom J Intern Med. 2008; 46:125-35.

Greenberg JA, Bell SJ, Guan Y, Yu YH. Folic Acid supplementation and pregnancy: more than just neural tube defect prevention. Rev Obstet Gynecol. 2011; 4:52-9.

Gröber U, Kisters K, Schmidt J. Neuroenhancement with vitamin B12-underestimated neurological significance. Nutrients. 2013; 5:5031-45.

Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. Thromb Res. 2004; 114:397-407.

Guilland JC, Aimone-Gastin I. Vitamin B9. Rev Prat. 2013; 63:1079, 1081-4.

Hackman DA, Farah MJ, Meaney MJ. Socioeconomic status and the brain: mechanistic insights from human and animal research. Nat Rev Neurosci. 2010; 11: 651-659.

Haddad J, Vilge V, Juif JG, Maitre M, Donato L, Messer J, Mark J. Beta-nerve growth factor levels in newborn cord sera. Pediatr Res. 1994; 35:637-9.

Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. Environ Health Perspect. 1994; 102 Suppl 10:5-12.

Hallows SE, Regnault TR, Betts DH. The long and short of it: the role of telomeres in fetal origins of adult disease. J Pregnancy. 2012; 2012:638476.

Hannan NJ, Evans J, Salamonsen LA. Alternate roles for immune regulators: establishing endometrial receptivity for implantation. Expert Rev Clin Immunol. 2011; 7:789-802.

Hanson M, Gluckman P. Developmental origins of noncommunicable disease: population and public health implications. Am J Clin Nutr. 2011; 94:1754S-8S

Hanson M, Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. Prog Biophys Mol Biol. 2011; 106:272-80.

Haram K, Mortensen JH, Nagy B. Genetic aspects of preeclampsia and the HELLP syndrome. J Pregnancy. 2014; 2014:910751.

Haram K, Svendsen E, Abildgaard U. The HELLP syndrome: clinical issues and management. A Review. BMC Pregnancy Childbirth. 2009; 9:8.

Health Quality Ontario. Vitamin B_{12} and cognitive function: an evidence-based analysis. Ont Health Technol Assess Ser. 2013; 13:1-45.

Hempstock J, Jauniaux E, Greenwold N, Burton GJ. The contribution of placental oxidative stress to early pregnancy failure. Hum Pathol. 2003; 34:1265-75.

Hennigan A, Callaghan CK, Kealy J, Rouine J, Kelly AM. Deficits in LTP and recognition memory in the genetically hypertensive rat are associated with decreased expression of neurotrophic factors and their receptors in the dentate gyrus. Behav Brain Res. 2009; 197:371-7.

Herrera EA, Krause B, Ebensperger G, Reyes RV, Casanello P, Parra-Cordero M, Llanos AJ. The placental pursuit for an adequate oxidant balance between the mother and the fetus. Front Pharmacol. 2014; 24;5:149.

Herrera-Garcia G, Contag S. Maternal preeclampsia and risk for cardiovascular disease in offspring. Curr Hypertens Rep. 2014; 16:475.

Herrmann W, Obeid R. Biomarkers of folate and vitamin B(12) status in cerebrospinal fluid. Clin Chem Lab Med. 2007; 45:1614-20.

Hill EP, Longo LD. Dynamics of maternal-fetal nutrient transfer. Fed Proc. 1980; 39:239-44.

Hill SY, Wang S, Carter H, Tessner K, Holmes B, McDermott M, Zezza N, Stiffler S. Cerebellum volume in high-risk offspring from multiplex alcohol dependence families: association with allelic variation in GABRA2 and BDNF. Psychiatry Res. 2011; 194:304-13.

Hirooka Y, Sagara Y, Kishi T, Sunagawa K. Oxidative stress and central cardiovascular regulation – Pathogenesis of hypertension and therapeutic aspects. Circ J. 2010; 74:827-35.

Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E, Huang T, Radziejewski C, Bailey K, Fandl JP,Daly T, Wiegand SJ, Yancopoulos GD, Rudge JS. VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci U S A. 2002; 99:11393-8.

Hoang HH, Padgham SV, Meininger CJ. L-arginine, tetrahydrobiopterin, nitric oxide and diabetes. Curr Opin Clin Nutr Metab Care. 2013; 16:76-82.

Hristova MG. Metabolic syndrome--from the neurotrophic hypothesis to a theory. Med Hypotheses. 2013; 81:627-34.

Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology. 2002; 180:33-44.

Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci. 2001; 24:677-736.

Hubel CA. Dyslipidemia, iron, and oxidative stress in preeclampsia: assessment of maternal and feto-placental interactions. Semin Reprod Endocrinol. 1998; 16:75-92.

Huppertz B, Berghold VM, Kawaguchi R, Gauster M. A variety of opportunities for immune interactions during trophoblast development and invasion. Am J Reprod Immunol. 2012; 67:349-57.

Huppertz B, Herrler A. Regulation of proliferation and apoptosis during development of the preimplantation embryo and the placenta. Birth Defects Res C Embryo Today. 2005; 75:249-61.

Huppertz B, Peeters LL. Vascular biology in implantation and placentation. Angiogenesis. 2005; 8:157-67.

Huppertz B, Weiss G, Moser G. Trophoblast invasion and oxygenation of the placenta: measurements versus presumptions. J Reprod Immunol. 2014; 101-102:74-9.

Hutcheon JA, Lisonkova S, Joseph KS. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. Best Pract Res Clin Obstet Gynaecol. 2011; 25:391-403.

Ikegame T, Bundo M, Murata Y, Kasai K, Kato T, Iwamoto K. DNA methylation of the BDNF gene and its relevance to psychiatric disorders. J Hum Genet. 2013; 58:434-8.

Ilekis JV, Reddy UM, Roberts JM. Preeclampsia--a pressing problem: an executive summary of a National Institute of Child Health and Human Development workshop. Reprod Sci. 2007; 14:508-23.

Imdad A, Yakoob MY, Bhutta ZA. The effect of folic acid, protein energy and multiple micronutrient supplements in pregnancy on stillbirths. BMC Public Health. 2011; 11:S4.

Jaatinen P, Rintala J. Mechanisms of ethanol-induced degeneration in the developing, mature, and aging cerebellum. Cerebellum. 2008; 7:332-47.

Jacobsen DW. Hyperhomocysteinemia and oxidative stress: time for a reality check? Arterioscler Thromb Vasc Biol. 2000; 20:1182-4.

James JL, Carter AM, Chamley LW. Human placentation from nidation to 5 weeks of gestation. Part I: What do we know about formative placental development following implantation? Placenta. 2012; 33:327-34.

Jansson T, Aye IL, Goberdhan DC. The emerging role of mTORC1 signaling in placental nutrient-sensing. Placenta. 2012; 33:e23-9.

Jansson T, Powell TL. Role of the placenta in fetal programming: underlying mechanisms and potential interventional approaches. Clin Sci (Lond). 2007; 113:1-13.

John R, Hemberger M. A placenta for life. Reprod Biomed Online. 2012; 25:5-11.

Jones ML, Mark PJ, Waddell BJ. Maternal dietary omega-3 fatty acids and placental function. Reproduction. 2014; 147:R143-52.

Kadyrov M, Moser G, Rath W, Kweider N, Wruck CJ, Pufe T, Huppertz BZ. Maternal-placental interactions and fetal programming. Geburtshilfe Neonatol. 2013; 217:88-94.

Kajantie E, Räikkönen K. Early life predictors of the physiological stress response later in life. Neurosci Biobehav Rev. 2010; 35:23-32.

Kalyanaraman B. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. Redox Biol. 2013; 1:244-57.

Kane SC, Dennis A, da Silva Costa F, Kornman L, Brennecke S. Contemporary clinical management of the cerebral complications of preeclampsia. Obstet Gynecol Int. 2013; 2013:985606.

Kapczinski F, Frey BN, Andreazza AC, Kauer-Sant'Anna M, Cunha AB, Post RM. Increased oxidative stress as a mechanism for decreased BDNF levels in acute manic episodes. Rev Bras Psiquiatr. 2008; 30:243-5.

Kar M. Role of biomarkers in early detection of preeclampsia. J Clin Diagn Res. 2014; 8:BE01-4.

Katoh-Semba R, Wakako R, Komori T, Shigemi H, Miyazaki N, Ito H, Kumagai T, Tsuzuki M, Shigemi K, Yoshida F, Nakayama A. Age-related changes in

BDNF protein levels in human serum: differences between autism cases and normal controls. Int J Dev Neurosci. 2007; 25:367-72.

Kawamura K, Kawamura N, Fukuda J, Kumagai J, Hsueh AJ, Tanaka T. Regulation of preimplantation embryo development by brain-derived neurotrophic factor. Dev Biol. 2007; 311:147-58.

Kawamura K, Kawamura N, Kumazawa Y, Kumagai J, Fujimoto T, Tanaka T. Brain-derived neurotrophic factor/tyrosine kinase B signaling regulates human trophoblast growth in an in vivo animal model of ectopic pregnancy. Endocrinology. 2011; 152:1090-100.

Kawamura K, Kawamura N, Sato W, Fukuda J, Kumagai J, Tanaka T. Brain-derived neurotrophic factor promotes implantation and subsequent placental development by stimulating trophoblast cell growth and survival. Endocrinology. 2009; 150:3774-82.

Kermani P, Hempstead B. Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. Trends Cardiovasc Med. 2007; 17:140-3.

Kermani P, Rafii D, Jin DK, Whitlock P, Schaffer W, Chiang A, Vincent L, Friedrich M, Shido K, Hackett NR, Crystal RG, Rafii S, Hempstead BL. Neurotrophins promote revascularization by local recruitment of TrkB+ endothelial cells and systemic mobilization of hematopoietic progenitors. J Clin Invest. 2005; 115:653-63.

Khan RN, Matharoo-Ball B, Shaw RW. Antioxidant enzyme expression, lipid peroxidation, and protein oxidation in human myometrium with parturition. Reprod Sci. 2010; 17:78-84.

Khot V, Chavan-Gautam P, Joshi S. Proposing interactions between maternal phospholipids and the one carbon cycle: A novel mechanism influencing the

risk for cardiovascular diseases in the offspring in later life. Life Sci. 2014 (In press)

Kilari A, Mehendale S, Pisal H, Panchanadikar T, Kale A, Joshi S. Nerve growth factor, birth outcome and pre-eclampsia. Int J Dev Neurosci. 2011; 29:71-5.

Kim H, Li Q, Hempstead BL, Madri JA. Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. J Biol Chem. 2004; 279:33538-46.

Kim JM, Hong K, Lee JH, Lee S, Chang N. Effect of folate deficiency on placental DNA methylation in hyperhomocysteinemic rats. J Nutr Biochem. 2009; 20:172-6.

Kim TS, Kim DJ, Lee H, Kim YK. Increased plasma brain-derived neurotrophic factor levels in chronic smokers following unaided smokingcessation. Neurosci Lett. 2007; 423:53-7.

Kimber SJ. Molecular interactions at the maternal-embryonic interface during the early phase of implantation. Semin Reprod Med. 2000;18(3):237-53.

Knöfler M. Critical growth factors and signalling pathways controlling human trophoblast invasion. Int J Dev Biol. 2010; 54:269-80.

Knüsel B, Rabin SJ, Hefti F, Kaplan DR. Regulated neurotrophin receptor responsiveness during neuronal migration and early differentiation.J Neurosci. 1994; 14:1542-54.

Kodomari I, Wada E, Nakamura S, Wada K. Maternal supply of BDNF to mouse fetal brain through the placenta. Neurochem Int. 2009; 54:95-8.

Koletzko B, Larqué E, Demmelmair H. Placental transfer of long-chain polyunsaturated fatty acids (LC-PUFA). J Perinat Med. 2007; 35:S5-11.

Kolling J, Scherer EB, da Cunha AA, da Cunha MJ, Wyse AT. Homocysteine induces oxidative-nitrative stress in heart of rats: prevention by folic acid. Cardiovasc Toxicol. 2011; 11:67-73.

Kräutler B. Biochemistry of B12-cofactors in human metabolism. Subcell Biochem. 2012; 56:323-46.

Kryzhanovskiĭ SA, Vititnova MB. Cardiovascular effects of nerve growth factor (analytical literature review) Part I. NGF-indirect intracellular signal pathways. Fiziol Cheloveka. 2011; 37:104-16.

Kulkarni A, Mehendale S, Pisal H, Kilari A, Dangat K, Salunkhe S, Taralekar V, Joshi S. Association of omega-3 fatty acids and homocysteine concentrations in pre-eclampsia. Clin Nutr. 2011; 30:60-4.

Kulkarni A, Dangat K, Kale A, Sable P, Chavan-Gautam P, Joshi S. Effects of altered maternal folic acid, vitamin B12 and docosahexaenoic acid on placental global DNA methylation patterns in Wistar rats. PLoS One. 2011; 6:e17706.

Kulkarni AV, Mehendale SS, Yadav HR, Joshi SR. Reduced placental docosahexaenoic acid levels associated with increased levels of sFlt-1 in preeclampsia. Prostaglandins Leukot Essent Fatty Acids. 2011; 84:51-5.

Kulkarni AV, Mehendale SS, Yadav HR, Kilari AS, Taralekar VS, Joshi SR. Circulating angiogenic factors and their association with birth outcomes in preeclampsia. Hypertens Res. 2010; 33:561-7.

Kumar N, Gupta N, Kishore J. Kuppuswamy's socioeconomic scale: updating income ranges for the year 2012. Indian J Public Health. 2012; 56: 103-104.

Kumar N. Neurologic aspects of cobalamin (B12) deficiency. Handb Clin Neurol. 2014; 120:915-26.

Kumar PR, Essa MM, Al-Adawi S, Dradekh G, Memon MA, Akbar M, Manivasagam T. Omega-3 Fatty acids could alleviate the risks of traumatic brain injury - a mini review. J Tradit Complement Med. 2014; 4:89-92.

LaMarca BD, Gilbert J, Granger JP. Recent progress toward the understanding of the pathophysiology of hypertension during preeclampsia. Hypertension. 2008; 51:982-8.

Langley-Evans SC. Developmental programming of health and disease. Proc Nutr Soc. 2006; 65:97-105.

Lappas M, Hiden U, Froehlich J, Desoye G, Haugel-de Mouzon S, Jawerbaum A. The Role of Oxidative Stress in the Pathophysiology of Gestational Diabetes Mellitus. Antioxid Redox Signal. Antioxid Redox Signal. 2011; 15:3061-100.

Larqué E, Gil-Sánchez A, Prieto-Sánchez MT, Koletzko B. Omega 3 fatty acids, gestation and pregnancy outcomes. Br J Nutr. 2012; 107:S77-84.

Larqué E, Ruiz-Palacios M, Koletzko B. Placental regulation of fetal nutrient supply. Curr Opin Clin Nutr Metab Care. 2013; 16:292-7.

Lash GE, Otun HA, Innes BA, Kirkley M, De Oliveira L, Searle RF, Robson SC, Bulmer JN. Interferon-gamma inhibits extravillous trophoblast cell invasion by a mechanism that involves both changes inapoptosis and protease levels. FASEB J. 2006; 20:2512-8.

Lasky-Su J, Faraone SV, Lange C, Tsuang MT, Doyle AE, Smoller JW, Laird NM, Biederman J. A study of how socioeconomic status moderates the relationship between SNPs encompassing BDNF and ADHD symptom counts in ADHD families. Behav Genet. 2007; 37:487-97.

Lazarovici P, Marcinkiewicz C, Lelkes PI. Cross talk between the cardiovascular and nervous systems: neurotrophic effects of vascular endothelial growth factor (VEGF) and angiogenic effects of nerve growth factor (NGF)-implications in drug development. Curr Pharm Des. 2006; 12:2609-22.

Lee JH. Polyunsaturated Fatty acids in children. Pediatr Gastroenterol Hepatol Nutr. 2013; 16:153-61.

Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. Science. 2001; 294:1945-8.

Lesseur C, Paquette AG, Marsit CJ. Epigenetic Regulation of Infant Neurobehavioral Outcomes. Med Epigenet. 2014; 2:71-79.

Li C, Zhou X. The potential roles of neurotrophins in male reproduction. Reproduction. 2013; 145:R89-95.

Li Q, Ford MC, Lavik EB, Madri JA. Modeling the neurovascular niche: VEGF- and BDNF-mediated cross-talk between neural stem cells andendothelial cells: an in vitro study. J Neurosci Res. 2006; 84:1656-68.

Lipps BV. Isolation of nerve growth factor (NGF) from human body fluids; saliva, serum and urine: comparison betweencobra venom and cobra serum NGF. J Nat Toxins. 2000; 9:349-56.

Lo JO, Mission JF, Caughey AB. Hypertensive disease of pregnancy and maternal mortality. Curr Opin Obstet Gynecol. 2013; 25:124-32.

Logan PC, Mitchell MD, Lobie PE. DNA methyltransferases and TETs in the regulation of differentiation and invasiveness of extra-villous trophoblasts. Front Genet. 2013; 4:265.

Loi M, Del Savio L, Stupka E. Social Epigenetics and Equality of Opportunity. Public Health Ethics. 2013; 6:142-153.

Lommatzsch M, Hornych K, Zingler C, Schuff-Werner P, Höppner J, Virchow JC. Maternal serum concentrations of BDNF and depression in the perinatal period. Psychoneuroendocrinology. 2006; 31:388-94.

Lommatzsch M, Zingler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-Werner P, Virchow JC. The impact of age, weight and gender on BDNF levels in human platelets and plasma. Neurobiol Aging. 2005; 26:115-23.

Longo FM, Massa SM. Small-molecule modulation of neurotrophin receptors: a strategy for the treatment of neurological disease. Nat Rev Drug Discov. 2013; 12:507-25.

Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. Neuron. 2002; 35:605-23.

López-Valladares MJ, Teresa Rodríguez-Ares M, Touriño R, Gude F, Teresa Silva M, Couceiro J. Donor age and gestational age influence on growth factor levels in human amniotic membrane. Acta Ophthalmol. 2010; 88:e211-6.

Lorgis L, Amoureux S, Vergely C, Zeller M, Cottin Y, Rochette L. Brain-Derived Neurotrophic Factor (BDNF): role of this neurotrophin in cardiovascular physiopathology. Ann Cardiol Angeiol (Paris). 2009; 58:99-103.

Lorquet S, Pequeux C, Munaut C, Foidart JM. Aetiology and physiopathology of preeclampsia and related forms. Acta Clin Belg. 2010; 65:237-41.

Lotfipour S, Ferguson E, Leonard G, Perron M, Pike B, Richer L, Séguin JR, Toro R, Veillette S, Pausova Z, Paus T. Orbitofrontal cortex and drug use during adolescence: role of prenatal exposure to maternal smoking and BDNF genotype. Arch Gen Psychiatry. 2009; 66:1244-52.

Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb Exp Pharmacol. 2014; 220:223-50.

Lubos E, Loscalzo J, Handy DE. Homocysteine and glutathione peroxidase-1. Antioxid Redox Signal. 2007; 9:1923-40.

Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab. 2000; 71:121-38.

Macbeth AH, Scharfman HE, Maclusky NJ, Gautreaux C, Luine VN. Effects of multiparity on recognition memory, monoaminergic neurotransmitters, and brain-derived neurotrophic factor (BDNF). Horm Behav. 2008; 54:7-17.

Maccani MA, Marsit CJ. Epigenetics in the placenta.Am J Reprod Immunol. 2009; 62:78-89.

Madan K, Babu AS, Contractor A, Sawhney JP, Prabhakaran D, Gupta R. Cardiac rehabilitation in India. Prog Cardiovasc Dis. 2014; 56:543-50.

Makrides M. The role of n-3 LCPUFA in pregnancy OCL 2011; 18: 255–8

Malamitsi-Puchner A, Economou E, Rigopoulou O, Boutsikou T. Perinatal changes of brain-derived neurotrophic factor in pre and fullterm neonates. Early Hum Dev. 2004; 76:17-22.

Malamitsi-Puchner A, Nikolaou KE, Economou E, Boutsikou M, Boutsikou T, Kyriakakou M, Puchner KP, Hassiakos D. Intrauterine growth restriction and circulating neurotrophin levels at term. Early Hum Dev. 2007; 83:465-9.

Malamitsi-Puchner A, Nikolaou KE, Puchner KP. Intrauterine growth restriction, brain-sparing effect, and neurotrophins. Ann N Y Acad Sci. 2006; 1092:293-6.

Manni L, Di Fausto V, Fiore M, Aloe L. Repeated restraint and nerve growth factor administration in male and female mice: effect on sympathetic and cardiovascular mediators of the stress response. Curr Neurovasc Res. 2008; 5:1-12.

Manni L, Rocco ML, Bianchi P, Soligo M, Guaragna M, Barbaro SP, Aloe L. Nerve growth factor: basic studies and possible therapeutic applications. Growth Factors. 2013; 31:115-22.

Mantovani E, Filippini F, Bortolus R, Franchi M. Folic acid supplementation and preterm birth: results from observational studies. Biomed Res Int. 2014; 2014:481914.

Many A, Fattal A, Leitner Y, Kupferminc MJ, Harel S, Jaffa A. Neurodevelopmental and cognitive assessment of children born growth restricted to mothers with and without preeclampsia. Hypertens Pregnancy. 2003; 22:25-9.

Mapp PI, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. Nat Rev Rheumatol. 2012; 8:390-8.

Marvin KW, Keelan JA, Eykholt RL, Sato TA, Mitchell MD. Expression of angiogenic and neurotrophic factors in the human amnion and choriodecidua. Am J Obstet Gynecol. 2002; 187:728-34.

Matsubara K,Higaki T, Matsubara Y, Nawa A. Nitric Oxide and Reactive Oxygen Species in the Pathogenesis of Preeclampsia. Int J Mol Sci. 2015; 16:4600-4614.

Marx CE, Vance BJ, Jarskog LF, Chescheir NC, Gilmore JH. Nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 levels in human amniotic fluid. Am J Obstet Gynecol. 1999; 181:1225-30.

Masi G, Brovedani P. The hippocampus, neurotrophic factors and depression: possible implications for the pharmacotherapy of depression. CNS Drugs. 2011; 25:913-31.

Matheson GO, Klügl M, Engebretsen L, Bendiksen F, Blair SN, Börjesson M, Budgett R, Derman W, Erdener U, Ioannidis JP, Khan KM, Martinez R, van

Mechelen W, Mountjoy M, Sallis RE, Schwellnus M, Shultz R, Soligard T, Steffen K, Sundberg CJ, Weiler R, Ljungqvist A. Prevention and management of noncommunicable disease: the IOC Consensus Statement, Lausanne 2013. Clin J Sport Med. 2013; 23:419-29.

Matoba N, Yu Y, Mestan K, Pearson C, Ortiz K, Porta N, Thorsen P, Skogstrand K, Hougaard DM, Zuckerman B, Wang X. Differential patterns of 27 cord blood immune biomarkers across gestational age. Pediatrics. 2009; 123:1320-8.

Mayeur S, Lukaszewski MA, Breton C, Storme L, Vieau D, Lesage J. Do neurotrophins regulate the feto-placental development? Med Hypotheses. 2011; 76:726-8.

Mayeur S, Silhol M, Moitrot E, Barbaux S, Breton C, Gabory A, Vaiman D, Dutriez-Casteloot I, Fajardy I, Vambergue A, Tapia-Arancibia L, Bastide B, Storme L,Junien C, Vieau D, Lesage J. Placental BDNF/TrkB signaling system is modulated by fetal growth disturbances in rat and human. Placenta. 2010; 31:785-91.

Mayhew TM, Charnock-Jones DS, Kaufmann P. Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. Placenta. 2004; 25:127-39.

Mayhew TM. Estimating oxygen diffusive conductances of gas-exchange systems: A stereological approach illustrated with the human placenta. Ann Anat. 2014; 196:34-40.

McCarthy DM, Brown AN, Bhide PG. Regulation of BDNF expression by cocaine. Yale J Biol Med. 2012; 85:437-46.

McKelvey L, Shorten GD, O'Keeffe GW. Nerve growth factor-mediated regulation of pain signalling and proposed new intervention strategies in clinical pain management. J Neurochem. 2013; 124:276-89.

Meeusen R. Exercise, nutrition and the brain. Sports Med. 2014; 44:S47-56.

Mehendale S, Kilari A, Dangat K, Taralekar V, Mahadik S, Joshi S. Fatty acids, antioxidants, and oxidative stress in pre-eclampsia. Int J Gynaecol Obstet. 2008; 100:234-8.

Meher AP, Joshi AA, Joshi SR. Preconceptional omega-3 fatty acid supplementation on a micronutrient-deficient diet improves the reproductive cycle in Wistar rats. Reprod Fertil Dev. 2013; 25:1085-94.

Mehndiratta MM, Singhal AB, Chaturvedi S, Sivakumar MR, Moonis M. Meeting the challenges of stroke in India. Neurology. 2013; 80:2246-7.

Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van Linthout S, Spillmann F, Campesi I, Madeddu P, Quaini F, Emanueli C. Nerve growth factor promotes cardiac repair following myocardial infarction. Circ Res. 2010; 106:1275-84.

Mess A, Carter AM. Evolution of the placenta during the early radiation of placental mammals. Comp Biochem Physiol A Mol Integr Physiol. 2007; 148:769-79.

Micle O, Muresan M, Antal L, Bodog F, Bodog A. The influence of homocysteine and oxidative stress on pregnancy outcome. J Med Life. 2012; 5:68-73.

Moffett-King. Natural killer cells and pregnancy. Nat Rev Immunol. 2002; 2:656-63.

Molendijk ML, Haffmans JP, Bus BA, Spinhoven P, Penninx BW, Prickaerts J, Oude Voshaar RC, Elzinga BM. Serum BDNF concentrations show strong seasonal variation and correlations with the amount of ambient sunlight. PLoS One. 2012; 7(11):e48046.

Molteni R, Lipska BK, Weinberger DR, Racagni G, Riva MA. Developmental and stress-related changes of neurotrophic factor gene expression in an animal model of schizophrenia. Mol Psychiatry. 2001; 6:285-92.

Moran P, Lindheimer MD, Davison JM. The renal response to preeclampsia. Semin Nephrol. 2004; 24:588-95.

Murray AJ. Oxygen delivery and fetal-placental growth: beyond a question of supply and demand? Placenta. 2012; 33:e16-22.

Murthi P. Review: placental homeobox genes and their role in regulating human fetal growth. Placenta. 2014; 35:S46-50.

Muskiet FA. The importance of (early) folate status to primary and secondary coronary artery disease prevention. Reprod Toxicol. 2005; 20:403-10.

Mutinati M, Piccinno M, Roncetti M, Campanile D, Rizzo A, Sciorsci R. Oxidative stress during pregnancy in the sheep. Reprod Domest Anim. 2013; 48:353-7.

Myatt L. Placental adaptive responses and fetal programming. J Physiol. 2006; 572:25-30.

Myatt L. Role of placenta in preeclampsia. Endocrine. 2002; 19:103-11.

Myatt L, Webster RP. Vascular biology of preeclampsia. J Throm Haemost. 2009; 7:375–84.

Nakamura K, Martin KC, Jackson JK, Beppu K, Woo CW, Thiele CJ. Brain-derived neurotrophic factor activation of TrkB induces vascular endothelial growth factor expression via hypoxia-inducible factor-1alpha in neuroblastoma cells. Cancer Res. 2006; 66:4249-55.

Nawa H, Bessho Y, Carnahan J, Nakanishi S, Mizuno K. Regulation of neuropeptide expression in cultured cerebral cortical neurons by brain-derived neurotrophic factor. J Neurochem. 1993; 60:772-5.

Nelissen EC, van Montfoort AP, Dumoulin JC, Evers JL. Epigenetics and the placenta. Hum Reprod Update. 2011; 17:397-417.

Nelson DB, Ziadie MS, McIntire DD, Rogers BB, Leveno KJ. Placental pathology suggesting that preeclampsia is more than one disease. Am J Obstet Gynecol. 2014; 210: 66.e1-7.

Neubert H, Clark TH, Deng S, Hurst SI. Serum β-nerve growth factor concentrations in pregnant female, nonpregnant female, and male cynomolgus monkeys. Neuroreport. 2014. (In press)

Nico B, Mangieri D, Benagiano V, Crivellato E, Ribatti D. Nerve growth factor as an angiogenic factor. Microvasc Res. 2008; 75:135-41.

Nieto R, Kukuljan M, Silva H. BDNF and schizophrenia: from neurodevelopment to neuronal plasticity, learning, and memory. Front Psychiatry. 2013; 4:45.

Nikolaou KE, Malamitsi-Puchner A, Boutsikou T, Economou E, Boutsikou M, Puchner KP, Baka S, Hassiakos D. The varying patterns of neurotrophin changes in the perinatal period. Ann N Y Acad Sci. 2006; 1092:426-33.

Noble EE, Billington CJ, Kotz CM, Wang C. The lighter side of BDNF. Am J Physiol Regul Integr Comp Physiol. 2011; 300:R1053-69.

Norris W, Nevers T, Sharma S, Kalkunte S. Review: hCG, preeclampsia and regulatory T cells. Placenta. 2011; 32 Suppl 2:S182-5.

Norwitz ER. Defective implantation and placentation: laying the blueprint for pregnancy complications. Reprod Biomed Online. 2007; 1:101-9.

Nowacka M, Obuchowicz E. BDNF and VEGF in the pathogenesis of stress-induced affective diseases: an insight from experimental studies. Pharmacol Rep. 2013; 65:535-46.

Oda M, Shiota K, Tanaka S. Trophoblast stem cells. Methods Enzymol. 2006; 419:387-400.

Ogunshola OO, Antic A, Donoghue MJ, Fan SY, Kim H, Stewart WB, Madri JA, Ment LR. Paracrine and autocrine functions of neuronal vascular endothelial growth factor (VEGF) in the central nervous system. J Biol Chem. 2002; 277:11410-5.

Ong S. In: Baker PN, Kingdom JCP, editors. Pr-eclampsia: Current perspectives on management. NY: The Parthenon Publishing Group. Pre-eclampsia: A historical perspective. 2004; 15-24.

Osungbade KO, Ige OK. Public health perspectives of preeclampsia in developing countries: implication for health system strengthening. J Pregnancy. 2011; 2011:481095.

Pardon MC. Role of neurotrophic factors in behavioral processes: implications for the treatment of psychiatric and neurodegenerative disorders. Vitam Horm. 2010; 82:185-200.

Parikh SM, Karumanchi SA. Putting pressure on pre-eclampsia. Nat Med. 2008; 14:810-2.

Park H, Poo MM. Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci. 2013; 14:7-23.

Park HR, Park M, Choi J, Park KY, Chung HY, Lee J. A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. Neurosci Lett. 2010; 482:235-9.

Park MJ, Kwak HJ, Lee HC, Yoo DH, Park IC, Kim MS, Lee SH, Rhee CH, Hong SI. Nerve growth factor induces endothelial cell invasion and cord formation by promoting matrix metalloproteinase-2 expression through the

phosphatidylinositol 3-kinase/Akt signaling pathway and AP-2 transcription factor. J Biol Chem. 2007; 282:30485-96.

Passaro A, Dalla Nora E, Morieri ML, Soavi C, Sanz JM, Zurlo A, Fellin R, Zuliani G. Brain-Derived Neurotrophic Factor Plasma Levels: Relationship with Dementia and Diabetes in the Elderly Population. J Gerontol A Biol Sci Med Sci. 2014 (in press).

Patapoutian A, Reichardt LF. Trk receptors: mediators of neurotrophin action. Curr Opin Neurobiol. 2001; 11:272-80.

Peet M. Nutrition and schizophrenia: beyond omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2004; 70:417-22.

Petit F, Huicq S, Gardette R, Epelbaum J, Loudes C, Kordon C, Faivre-Bauman A. The neurotrophins NT3 and BDNF induce selective specification of neuropeptide coexpression and neuronal connectivity in arcuate and periventricular hypothalamic neurons in vitro. Neuroendocrinology. 2002; 75:55-69.

Petla LT, Chikkala R, Ratnakar KS, Kodati V, Sritharan V. Biomarkers for the management of pre-eclampsia in pregnant women. Indian J Med Res. 2013; 138:60-7.

Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, van Assche A. Placental bed spiral arteries in the hypertensive disorders of pregnancy. Br J Obstet Gynaecol. 1991; 98:648-55.

Pillai A, Bruno D, Sarreal AS, Hernando RT, Saint-Louis LA, Nierenberg J, Ginsberg SD, Pomara N, Mehta PD, Zetterberg H, Blennow K, Buckley PF. Plasma BDNF levels vary in relation to body weight in females. PLoS One. 2012; 7:e39358.

Pillai A, Kale A, Joshi S, Naphade N, Raju MS, Nasrallah H, Mahadik SP. Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology. Int J Neuropsychopharmacol. 2010; 13:535-9.

Pinheiro KA, Pinheiro RT, Coelho FM, da Silva RA, Quevedo LÁ, Schwanz CC, Wiener CD, Manfro GG, Giovenardi M, Lucion AB, de Souza DO, Portela LV, Oses JP. Serum NGF, BDNF and IL-6 levels in postpartum mothers as predictors of infant development: the influence of affective disorders. PLoS One. 2014; 9:e94581.

Pinheiro RT, Pinheiro KA, da Cunha Coelho FM, de Ávila Quevedo L, Gazal M, da Silva RA, Giovenardi M, Lucion AB, de Souza DO, Portela LV, Oses JP. Brain-derived neurotrophic factor levels in women with postpartum affective disorder and suicidality. Neurochem Res. 2012; 37:2229-34.

Poljsak B. Strategies for reducing or preventing the generation of oxidative stress. Oxid Med Cell Longev. 2011; 2011:194586.

Pollack SJ, Harper SJ. Trk Neurotrophin Receptor Activators. Drug News Perspect. 2002; 15:268-277.

Pollheimer J, Knöfler M. The role of the invasive, placental trophoblast in human pregnancy. Wien Med Wochenschr. 2012; 162:187-90.

Poston L, Igosheva N, Mistry HD, Seed PT, Shennan AH, Rana S, Karumanchi SA, Chappell LC. Role of oxidative stress and antioxidant supplementation in pregnancy disorders. Am J Clin Nutr. 2011 (In press)

Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. Circulation. 2011; 123:2856-69.

Pringle KG, Kind KL, Sferruzzi-Perri AN, Thompson JG, Roberts CT. Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy. Hum Reprod Update. 2010; 16:415-31.

Purcell WM, Atterwill CK. Human placental mast cells as an in vitro model system in aspects of neuro-immunotoxicity testing. Hum Exp Toxicol. 1994; 13:429-33.

Qiu C, Sanchez SE, Lam N, Garcia P, Williams MA. Associations of depression and depressive symptoms with preeclampsia: results from a Peruvian case-control study. BMC Womens Health. 2007; 7:15.

Quinn M. Pre-eclampsia and partial uterine denervation. Med Hypotheses. 2005; 64:449-54.

Rachaneni S, Arya P, Latthe P. Urinary nerve growth factor: a biomarker of detrusor overactivity? A systematic review. Int Urogynecol J. 2013; 24:1603-9.

Raghupathy R. Cytokines as key players in the pathophysiology of preeclampsia. Med Princ Pract. 2013; 22:8-19.

Rahman K. Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging. 2007; 2:219-36.

Rai A, Cross JC. Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. Dev Biol. 2014; 387:131-41.

Ram K, Santoro N. Does pregnancy-induced hypertension increase the risk of developing metabolic syndrome? Nat Clin Pract Endocrinol Metab. 2005; 1:76-7.

Ramírez-Vélez R, Romero M, Echeverri I, Ortega JG, Mosquera M, Salazar B, Girón SL, Saldarriaga W, Aguilar de Plata AC, Mateus JC. A factorial randomized controlled trial to evaluate the effect of micronutrients supplementation and

regular aerobicexercise on maternal endothelium-dependent vasodilatation and oxidative stress of the newborn. Trials. 2011; 12:60.

Rao R, Mashburn CB, Mao J, Wadhwa N, Smith GM, Desai NS. Brain-derived neurotrophic factor in infants <32 weeks gestational age: correlation with antenatal factors and postnatal outcomes. Pediatr Res. 2009; 65:548-52.

Rathod R, Khaire A, Kemse N, Kale A, Joshi S. Maternal omega-3 fatty acid supplementation on vitamin B12 rich diet improves brain omega-3 fatty acids, neurotrophins and cognition in the Wistar rat offspring. Brain Dev. 2014; 36:853-63.

Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: making sense of pre-eclampsia - two placental causes of preeclampsia? Placenta. 2014; 35:S20-5.

Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005; 308:1592-4.

Redman CW. Current topic: pre-eclampsia and the placenta. Placenta. 1991; 12:301-8.

Rehn AE, Rees SM. Investigating the neurodevelopmental hypothesis of schizophrenia. Clin Exp Pharmacol Physiol. 2005; 32:687-96.

Rehn AE, Van Den Buuse M, Copolov D, Briscoe T, Lambert G, Rees S. An animal model of chronic placental insufficiency: relevance to neurodevelopmental disorders including schizophrenia. Neuroscience. 2004; 129:381-91.

Reichardt LF. Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci. 2006; 361:1545-64.

Reynolds LP, Borowicz PP, Caton JS, Vonnahme KA, Luther JS, Hammer CJ, Maddock Carlin KR, Grazul-Bilska AT, Redmer DA. Developmental

programming: the concept, large animal models, and the key role of uteroplacental vascular development. J Anim Sci. 2010; 88:E61-72.

Reynolds LP, Redmer DA. Angiogenesis in the placenta. Biol Reprod. 2001; 64:1033-40.

Ricci A, Bronzetti E, Mannino F, Felici L, Terzano C, Mariotta S. Elevated neurotrophin and neurotrophin receptor expression in spontaneously hypertensive rat lungs. Growth Factors. 2004; 22:195-205.

Rios M. Neurotrophins and the regulation of energy balance and body weight. Handb Exp Pharmacol. 2014; 220:283-307.

Roberti G, Mantelli F, Macchi I, Massaro-Giordano M, Centofanti M. Nerve growth factor modulation of retinal ganglion cell physiology. J Cell Physiol. 2014; 229:1130-3.

Roberts JM, Bell MJ. If we know so much about preeclampsia, why haven't we cured the disease? J Reprod Immunol. 2013; 99:1-9.

Roberts JM, Catov JM. Preeclampsia more than 1 disease: or is it? Hypertension. 2008; 51:989-90.

Rosa BV, Blair HT, Vickers MH, Dittmer KE, Morel PC, Knight CG, Firth EC. Moderate exercise during pregnancy in Wistar rats alters bone and body composition of the adult offspring in a sex-dependent manner. PLoS One. 2013; 8:e82378.

Rosas-Vargas H, Martínez-Ezquerro JD, Bienvenu T. Brain-derived neurotrophic factor, food intake regulation, and obesity. Arch Med Res. 2011; 42:482-94.

Rosser ML, Katz NT. Preeclampsia: an obstetrician's perspective. Adv Chronic Kidney Dis. 2013; 20:287-96.

Roten LT, Fenstad MH, Forsmo S, Johnson MP, Moses EK, Austgulen R, Skorpen F. A low COMT activity haplotype is associated with recurrent preeclampsia in a Norwegian population cohort (HUNT2). Mol Hum Reprod. 2011; 17:439-46.

Roth TL, Sweatt JD. Annual Research Review: Epigenetic mechanisms and environmental shaping of the brain during sensitiveperiods of development. J Child Psychol Psychiatry. 2011; 52:398-408.

Roura LC, Arulkumaran SS. Facing the noncommunicable disease (NCD) global epidemic - The battle of prevention starts in utero - The FIGO challenge. Best Pract Res Clin Obstet Gynaecol. 2014 (In press)

Rush EC, Katre P, Yajnik CS. Vitamin B12: one carbon metabolism, fetal growth and programming for chronic disease. Eur J Clin Nutr. 2014; 68:2-7.

Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol. 2009 ; 77:937-46.

Sable PS, Kale AA, Joshi SR. Prenatal omega 3 fatty acid supplementation to a micronutrient imbalanced diet protects brain neurotrophins in both the cortex and hippocampus in the adult rat offspring. Metabolism. 2013; 62:1607-22.

Sado T, Naruse K, Noguchi T, Haruta S, Yoshida S, Tanase Y, Kitanaka T, Oi H, Kobayashi H. Inflammatory pattern recognition receptors and their ligands: factors contributing to the pathogenesis of preeclampsia. Inflamm Res. 2011; 60:509-20.

Sakamoto K, Karelina K, Obrietan K. CREB: a multifaceted regulator of neuronal plasticity and protection. J Neurochem. 2011; 116:1-9.

Savaj S, Vaziri N. An overview of recent advances in pathogenesis and diagnosis of preeclampsia. Iran J Kidney Dis. 2012; 6:334-8.

Schanker BD. Epigenetic regulation of brain-derived neurotrophic factor: implications in neurodevelopment and behavior. Behav Brain Sci. 2012; 35:377-8.

Scharfman HE, MacLusky NJ. Estrogen-growth factor interactions and their contributions to neurological disorders. Headache. 2008; 48 Suppl 2:S77-89.

Schneider H. Oxygenation of the placental-fetal unit in humans. Respir Physiol Neurobiol. 2011; 178:51-8.

Schnorr CE1, Morrone Mda S, Weber MH, Lorenzi R, Behr GA, Moreira JC. The effects of vitamin A supplementation to rats during gestation and lactation upon redox parameters: increased oxidative stress and redox modulation in mothers and their offspring. Food Chem Toxicol. 2011; 49:2645-54.

Sears ME, Genuis SJ. Environmental determinants of chronic disease and medical approaches: recognition, avoidance, supportive therapy, and detoxification. J Environ Public Health. 2012; 2012:356798.

Sharma S, Zhuang Y, Gomez-Pinilla F. High-fat diet transition reduces brain DHA levels associated with altered brain plasticity and behaviour. Sci Rep. 2012; 2:431.

Sharp AN, Heazell AE, Crocker IP, Mor G. Placental apoptosis in health and disease. Am J Reprod Immunol. 2010; 64:159-69.

Sher L, La Bode V. Childhood abuse, brain-derived neurotrophic factor and psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2011; 13:19–20.

Shoval G, Weizman A. The possible role of neurotrophins in the pathogenesis and therapy of schizophrenia. Eur Neuropsychopharmacol. 2005; 15:319-29.

Sibai BM. Thrombophilia and severe preeclampsia: time to screen and treat in future pregnancies? Hypertension. 2005; 46:1252-3.

Silveira PP, Portella AK, Goldani MZ, Barbieri MA. Developmental origins of health and disease (DOHaD). J Pediatr (Rio J). 2007; 83:494-504.

Sinclair KD, Singh R. Modelling the developmental origins of health and disease in the early embryo. Theriogenology. 2007; 67:43-53.

Skaper SD. The neurotrophin family of neurotrophic factors: an overview. Methods Mol Biol. 2012; 846:1-12.

Sofroniew MV, Howe CL, Mobley WC. Nerve growth factor signaling, neuroprotection, and neural repair. Annu Rev Neurosci. 2001; 24:1217-81.

Solomonson LP, Flam BR, Pendleton LC, Goodwin BL, Eichler DC. The caveolar nitric oxide synthase/arginine regeneration system for NO production in endothelial cells. J Exp Biol. 2003; 206:2083-7.

Sones JL, Lob HE, Isroff CE, Davisson RL. Role of decidual natural killer cells, interleukin-15, and interferon-γ in placental development and preeclampsia. Am J Physiol Regul Integr Comp Physiol. 2014; 307:R490-R492.

Sopova K, Gatsiou K, Stellos K, Laske C. Dysregulation of neurotrophic and haematopoietic growth factors in Alzheimer's disease: from pathophysiology to novel treatment strategies. Curr Alzheimer Res. 2014; 11:27-39.

Soto E, Romero R, Kusanovic JP, Ogge G, Hussein Y, Yeo L, Hassan SS, Kim CJ, Chaiworapongsa TJ. Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal underperfusion. Matern Fetal Neonatal Med. 2012; 25:498-507.

Srinivas SK, Morrison AC, Andrela CM, Elovitz MA. Allelic variations in angiogenic pathway genes are associated with preeclampsia. Am J Obstet Gynecol. 2010; 202:445.e1-11.

Stark MJ, Clifton VL, Hodyl NA. Differential effects of docosahexaenoic acid on preterm and term placental pro-oxidant/antioxidant balance. Reproduction. 2013 146:243-51.

Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest. 1986; 77:1370-6.

Steinert JR, Wyatt AW, Jacob R, Mann GE. Redox modulation of Ca2+ signaling in human endothelial and smooth muscle cells in pre-eclampsia. Antioxid Redox Signal. 2009; 11:1149-63.

Subramaniam V. Seasonal variation in the incidence of preeclampsia and eclampsia in tropical climatic conditions. BMC Womens Health. 2007; 7:18.

Suganuma H, Arai Y, Kitamura Y, Hayashi M, Okumura A, Shimizu T. Maternal docosahexaenoic acid-enriched diet prevents neonatal brain injury. Neuropathology. 2010; 30:597-605.

Sundrani D, Khot V, Pisal H, Mehendale S, Wagh G, Joshi A, Joshi S. Gestation dependant changes in angiogenic factors and their associations with fetal growth measures innormotensive pregnancy. PLoS One. 2013; 8:e54153.

Sundrani DP, Chavan Gautam PM, Mehendale SS, Joshi SR. Altered metabolism of maternal micronutrients and omega 3 fatty acids epigenetically regulate matrix metalloproteinases in preterm pregnancy: a novel hypothesis. Med Hypotheses. 2011; 77:878-83.

Supowit SC, Zhao H, DiPette DJ. Nerve growth factor enhances calcitonin gene-related peptide expression in the spontaneously hypertensive rat. Hypertension. 2001; 37:728-32.

Syed Z, Dudbridge F, Kent L. An investigation of the neurotrophic factor genes GDNF, NGF, and NT3 in susceptibility to ADHD. Am J Med Genet B Neuropsychiatr Genet. 2007; 144B:375-8.

Tadesse S, Kidane D, Guller S, Luo T, Norwitz NG, Arcuri F, Toti P, Norwitz ER. In vivo and in vitro evidence for placental DNA damage in preeclampsia. PLoS One. 2014; 9:e86791.

Talaulikar VS, Manyonda IT. Vitamin C as an antioxidant supplement in women's health: a myth in need of urgent burial. Eur J Obstet Gynecol Reprod Biol. 2011; 157:10-3.

Tamura T, Picciano MF. Folate and human reproduction.Am J Clin Nutr. 2006; 83:993-1016.

Telang MA, Bhutkar SP, Hirwani RR. Analysis of patents on preeclampsia detection and diagnosis: a perspective. Placenta. 2013; 34:2-8.

Teng KK, Felice S, Kim T, Hempstead BL. Understanding proneurotrophin actions: Recent advances and challenges. Dev Neurobiol. 2010; 70:350-9.

Tessarollo L. Pleiotropic functions of neurotrophins in development. Cytokine Growth Factor Rev. 1998; 9:125-37.

Thaler CJ. Folate Metabolism and Human Reproduction. Geburtshilfe Frauenheilkd. 2014; 74:845-851.

The American College of Obstetricians and Gynecologists (ACOG) 2014. Annual Clinical Meeting. Chicago, Illinois, USA.

Thiele CJ, Li Z, McKee AE. On Trk--the TrkB signal transduction pathway is an increasingly important target in cancer biology. Clin Cancer Res. 2009; 15:5962-7.

Thoenen H, Barde YA, Davies AM, Johnson JE. Neurotrophic factors and neuronal death. Ciba Found Symp. 1987; 126:82-95.

Torry DS, Hinrichs M, Torry RJ. Determinants of placental vascularity. Am J Reprod Immunol. 2004; 51:257-68.

Toti P, Ciarmela P, Florio P, Volpi N, Occhini R, Petraglia F. Human placenta and fetal membranes express nerve growth factor mRNA and protein. J Endocrinol Invest. 2006; 29:337-41.

Tousoulis D, Briasoulis A, Papageorgiou N, Tsioufis C, Tsiamis E, Toutouzas K, Stefanadis C. Oxidative stress and endothelial function: therapeutic interventions. Recent Pat Cardiovasc Drug Discov. 2011; 6:103-14.

Turk-Adawi K, Sarrafzadegan N, Grace SL. Global availability of cardiac rehabilitation. Nat Rev Cardiol. 2014 (In press)

Tyagi E, Agrawal R, Zhuang Y, Abad C, Waschek JA, Gomez-Pinilla F. Vulnerability imposed by diet and brain trauma for anxiety-like phenotype: implications for post-traumatic stress disorders. PLoS One. 2013; 8:e57945.

Umhau JC, Dauphinais KM, Patel SH, Nahrwold DA, Hibbeln JR, Rawlings RR, George DT. The relationship between folate and docosahexaenoic acid in men. Eur J Clin Nutr. 2006; 60:352-7.

van Dijk M, Oudejans CB. STOX1: Key player in trophoblast dysfunction underlying early onset preeclampsia with growth retardation. J Pregnancy. 2011; 2011:521826.

van Patot MC, Ebensperger G, Gassmann M, Llanos AJ. The hypoxic placenta. High Alt Med Biol. 2012;13:176-84.

van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci. 2000; 1:191-8.

Varendi K, Kumar A, Härma MA, Andressoo JO. miR-1, miR-10b, miR-155, and miR-191 are novel regulators of BDNF. Cell Mol Life Sci. 2014. (In press)

Vega SR1, Kleinert J, Sulprizio M, Hollmann W, Bloch W, Strüder HK. Responses of serum neurotrophic factors to exercise in pregnant and postpartum women. Psychoneuroendocrinology. 2011; 36:220-7.

Vicario-Abejón C, Owens D, McKay R, Segal M. Role of neurotrophins in central synapse formation and stabilization. Nat Rev Neurosci. 2002; 3:965-74.

Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med. 2009; 27:358-68.

Wadhwani N, Patil V, Pisal H, Joshi A, Mehendale S, Gupte S, Wagh G, Joshi S. Altered maternal proportions of long chain polyunsaturated fatty acids and their transport leads to disturbed fetal stores in preeclampsia. Prostaglandins Leukot Essent Fatty Acids. 2014; 91:21-30.

Wahlqvist ML. Antioxidant relevance to human health. Asia Pac J Clin Nutr. 2013; 22:171-6.

Wareing M. Oxygen sensitivity, potassium channels, and regulation of placental vascular tone. Microcirculation. 2014; 21:58-66.

Watanabe F, Yabuta Y, Tanioka Y, Bito T. Biologically active vitamin B12 compounds in foods for preventing deficiency among vegetarians and elderly subjects. J Agric Food Chem. 2013; 61:6769-75.

Weaver IC. Epigenetic programming by maternal behavior and pharmacological intervention. Nature versus nurture: let's call the whole thing off. Epigenetics. 2007; 2:22-8.

Weiss G, Goldsmith LT, Taylor RN, Bellet D, Taylor HS. Inflammation in reproductive disorders. Reprod Sci. 2009; 16:216-29.

Wu A, Ying Z, Gomez-Pinilla F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disabilityafter traumatic brain injury in rats. J Neurotrauma. 2004a; 21:1457-67.

Wu A, Ying Z, Gomez-Pinilla F. Docosahexaenoic acid dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition. Neuroscience. 2008; 55:751-9.

Wu A, Ying Z, Gomez-Pinilla F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of saturated fat diet on synaptic plasticity and cognition. Eur J Neurosci. 2004b; 19:1699-707.

Xia Y, Wen H, Bobst S, Day MC, Kellems RE. Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human trophoblast cells. J Soc Gynecol Investig. 2003; 10:82-93.

Yan J, Yang H. Gestational diabetes mellitus, programing and epigenetics. J Matern Fetal Neonatal Med. 2014; 27:1266-9.

Yang X, Guo L, Li H, Chen X, Tong X. Analysis of the original causes of placental oxidative stress in normal pregnancy and pre-eclampsia: a hypothesis. J Matern Fetal Neonatal Med. 2012; 25:884-8.

Xia XY, Huang XH, Xia YX, Zhang WH. Changes of nerve growth factor in amniotic fluid and correlation with ventriculomegaly. Chin Med Sci J. 2011; 26:109-12.

Yelumalai S, Muniandy S, Zawiah Omar S, Qvist R. Pregnancy-induced hypertension and preeclampsia: levels of angiogenic factors in malaysian women. J Clin Biochem Nutr. 2010; 47:191-7.

Yochum C, Doherty-Lyon S, Hoffman C, Hossain MM, Zelikoff JT, Richardson JR. Prenatal cigarette smoke exposure causes hyperactivity and aggressive behavior: role of altered catecholamines and BDNF. Exp Neurol. 2014; 254:145-52.

Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. Annu Rev Pathol. 2010; 5:173-92.

Zdanys KF, Kleiman TG, Zhang H, Ozbay F, MacAvoy MG, Gelernter J, van Dyck CH. BDNF variants, premorbid educational attainment, and disease characteristics in Alzheimer's disease: an exploratory study. J Alzheimers Dis. 2009; 17:887-98.

Zettler C, Bridges DC, Zhou XF, Rush RA. Detection of increased tissue concentrations of nerve growth factor with an improved extraction procedure. J Neurosci Res. 1996; 46:581-94.

Zhang Q, Chen Q, Lu X, Zhou Z, Zhang H, Lin HY, Duan E, Zhu C, Tan Y, Wang H. CUL1 promotes trophoblast cell invasion at the maternal-fetal interface. Cell Death Dis. 2013; 4:e502.

Zuniga, Ormazabal V, Gutierrez N, Aguilera V, Radojkovic C, Veas C, Escudero C, Lamperti L, Aguayo C. Role of lectin-like oxidized low density lipoprotein-1 in fetoplacental vascular dysfunction in preeclampsia. Biomed Res Int. 2014; 2014:353616.

PUBLICATIONS

PUBLICATIONS

Total No. 3

Total Impact Factor = 8.812 Average Impact Factor = 2.937

1. **D'Souza V**, Patil V, Pisal H, Randhir K, Joshi A, Mehendale S, Wagh G, Gupte S, Joshi S. (2014) Levels of brain derived neurotrophic factors across gestation in women with preeclampsia. ***International Journal of Developmental Neuroscience*** 37:36-40 *(Impact Factor: 2.918)*

2. **D'Souza V**, Kilari A, Joshi A, Mehendale S, Pisal H, Joshi S. (2014) Differential regulation of brain-derived neurotrophic factor in term and preterm preeclampsia. ***Reproductive Sciences*** 21:230-5 *(Impact Factor: 2.179)*

3. **D'Souza V**, Chavan-Gautam P, Joshi S. (2013) Counteracting oxidative stress in pregnancy through modulation of maternal micronutrients and omega-3 fatty acids. ***Current Medicinal Chemistry*** 20:4777-83 *(Impact Factor: 3.715)*

PUBLICATIONS (Communicated)

1. **D'Souza V**, Rani A, Patil V, Pisal H, Randhir K, Joshi A, Mehendale S, Wagh G, Gupte S, Joshi S. Increased oxidative stress from early pregnancy in women who develop preeclampsia. (***The Scandinavian Journal of Clinical and Laboratory Investigation***)

2. **D'Souza V**, Kilari A, Patil V, Pisal H, Randhir K, Joshi A, Mehendale S, Wagh G, Gupte S, Joshi S. Maternal nerve growth factor levels in women with preeclampsia: a longitudinal study. (***International Journal of Developmental Neuroscience***)

AWARD

AWARD

Total No. 1

REGISTRATION AND TRAVEL AWARD for a Young Scientist by Indian Council of Medical Research for the paper entitled "Maternal Nerve Growth Factor Levels in Women with Preeclampsia from Two Socioeconomic Groups: A Longitudinal Study" at the 8th World Congress on Developmental Origins of Health and Disease, Singapore, 17th – 20th November 2013.

INTERNATIONAL CONFERENCES

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Total No. 3

1. **D'Souza V**, Mehendale S, Wagh G, Gupte S, Patil V, Pisal H, Dhobale M and Joshi S. Altered levels of antioxidant enzymes and oxidative stress marker throughout gestation in women with preeclampsia. **Recent Trends in Free Radical and Antioxidant Research & Thirteenth Annual Meeting of the Society for Free Radical Research,** Lonavala, Maharashtra, India, 27th – 30th January, 2014.

2. **D'Souza V**, Mehendale S, Wagh G, Gupte S, Patil V, Pisal H, Dhobale M and Joshi S. Maternal nerve growth factor levels in women with preeclampsia from two socioeconomic groups: a longitudinal study. **8th World Congress on Developmental Origins of Health and Disease**, Singapore, 17th – 20st November, 2013.

3. **D'Souza V**, Kilari A, Mehendale S, Pisal H, Joshi S. Brain derived neurotrophic factor levels in preeclampsia. **7th World Congress on Developmental Origins of Health and Disease**, Portland, Oregon USA, 18th -21st September, 2011.

NATIONAL CONFERENCES

NATIONAL CONFERENCES

Total No. 3

1. **D'Souza V**, Mehendale S, Wagh G, Gupte S, Patil V, Pisal H, Joshi S. Brain Derived Neurotrophic Factor Levels in Preeclampsia (Oral Presentation – Free Communication), at the **44th National Conference of the Nutrition Society of India, Tirupati,** 16th- 17th November, 2012. OPEN-3.

2. **D'Souza V**, Kilari A, Joshi A, Pisal H. and Joshi S. Differential Regulation of Cord Brain Derived Neurotrophic Factor Levels in Term and Preterm Preeclampsia (Young Scientist – Junior Award), at the **43rd National Conference of the Nutrition Society of India, Hyderabad,** 11th- 12th November, 2011. JAEN-09.

3. Kulkarni A , **D'Souza V**, Mehendale S, Yadav H, Joshi S. Reduced Placental Docosahexaenoic Acid Levels Associated with Increased Plasma Soluble Flt-1 Levels in Preeclampsia (Oral Presentation – Free Communication), at the **42nd National Conference of the Nutrition Society of India, Mumbai,** 19th - 20th November, 2010. FC/CN/E.

WORKSHOP / COURSE / SEMINAR

WORKSHOP / COURSE / SEMINAR

Total No. 3

1. **1st David Barker Memorial Symposium** on the **Developmental Origins of Health and Disease** at the **18th Annual SNEHA-India International Workshop**, Fariyas Resort, Lonavala, India, 26th -28th September 2014.

2. **International Course in Nutrition Research Methods** organized and sponsored by the **Bangalore Boston Nutrition Collaborative (BBNC)** at St. John's Research Institute in Bangalore (SJRI) from 21st January –1st February, 2013.

3. ICMR centenary celebrations National Institute of Nutrition organized **Seminar (one day) / brain storming session (half a day) Maternal under-nutrition and developmental origins of health and disease held at NIN**, **Hyderabad,** 25th - 26th June, 2011.