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Maternal Micronutrients, Omega-3 Fatty Acids and Gene Expression of Angiogenic and Inflammatory Markers in Pregnancy Induced Hypertension Rats

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Background and Aims. Preeclampsia is a disorder of pregnancy and is associated with inflammation and altered angiogenesis. The present study examines the effect of micronutrient and omega-3 fatty acid supplementation (individual, as well as combined) on genes involved in inflammation and angiogenesis, as well as global DNA methylation levels in a pregnancy induced hypertension (PIH) rat model.

Methods. Pregnant Wistar rats were randomly assigned to six dietary groups: control, PIH (Pregnancy induced hypertension) Induced; PIH Induced with micronutrient supplements with vitamin B_{12} (PIHB), folate (PIHF), omega-3 fatty acid (PIHO), and combined supplementation (PIHC) (micronutrients and omega-3 fatty acids). Half the dams were dissected on 20 d of gestation to collect placental tissue, and half were allowed to deliver normally on 22 d of gestation and were assigned to a postnatal control diet. The offspring were dissected at 3 month of age.

Results. PIH induction increased the mRNA levels of the pro inflammatory cytokine IL-6 ($p < 0.01$), while lowering the placental anti inflammatory cytokine IL-10 ($p < 0.05$) at d20 of gestation. It also increased the expression of TNF-α ($p < 0.05$) in the liver of 3 month old offspring. The combined supplementation of folic acid, vitamin B_{12} and omega-3 fatty acids improved placental IL-10 levels and decreased TNF-α levels in offspring livers.

Conclusion. Our data indicate that a combined supplementation of vitamin B_{12}, folic acid and omega-3 fatty acid was useful for the better management of preeclampsia in an animal model.

Key Words: Docosahexaenoic acid, Folate, Inflammation, Pregnancy induced hypertension, Vitamin B_{12}.

Introduction

Preeclampsia (PE) is a multisystem disorder of pregnancy, which leads to maternal and neonatal morbidity and mortality (1). It originates in the placenta and is one of the most common causes of preterm birth and fetal growth restriction (2). It is believed that 'excessive inflammation' and 'angiogenic imbalance' is associated with PE (3–6). Maternal nutrition has been shown to influence the susceptibility of the offspring's genome to complex diseases in adulthood (7). Many of the acquired adverse effects are suggested to be a result of changes in placental DNA methylation patterns (8,9).

Epigenetic processes such as DNA methylation are known to regulate gene expression in a tissue-specific manner and induce long-term functional and metabolic changes which persist in later life (10,11). These epigenetic modifications are of major relevance in pregnancy complications like preeclampsia as it has been suggested that their children may be at an increased risk of developing high blood pressure in later life (12,13). Micronutrients such

Competing Interests: None declared.

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as folate and vitamin B_{12} are involved in the one carbon cycle and play a critical role in the supply of methyl groups for several methylation reactions (14). Studies also indicate the crucial role of fatty acids in mediating epigenetic mechanisms (9,15,16). We have previously demonstrated that micronutrients (vitamin B_{12}, folic acid) and omega-3 fatty acids are components of the one carbon cycle, and together may influence the placental methylation and the expression of fatty acid desaturases in the placenta (17,18). Our earlier study on human preeclampsia placenta indicates altered placental global DNA methylation levels as compared to normotensive placenta (19).

We have recently reported higher levels of inflammatory markers such as the tumor necrosis factor (TNF)-α (20) and lower levels of angiogenic vascular endothelial growth factor (VEGF) and anti-inflammatory marker like interleukin (IL)-10 in the placenta of the PIH rat model (21). It is likely that these changes in protein levels of angiogenic and inflammatory markers could be a result of altered placental DNA methylation levels influencing gene expression levels.

In our recent paper (21), we only reported interleukin IL-6 and IL-10 protein levels. It is likely that these changes in protein levels could be a result of altered placental DNA methylation levels influencing the gene expression levels. This manuscript reports IL-6 and IL-10 mRNA levels. Also reported are placental mRNA levels of TNF-α and global DNA methylation levels. It is likely that expression patterns may persist in adult life. The offspring were therefore followed up at 3 month of age to examine the mRNA levels of IL-6, IL-10, TNF-α, VEGF and liver vascular endothelial growth factor receptor-1 (VEGFR-1).

Materials and Methods

This study followed the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experimental Animals of the government of India. Ethical approval was provided by the Institutional Animal Ethical Committee (IAEC/CPCSEA/2311) of Bharati Vidyapeeth.

Animals and Breeding

Pregnant Wistar albino rats (n = 16 per group) were randomly assigned to six dietary groups which included control, PIH (Pregnancy induced hypertension) Induced, PIH Induced with vitamin B_{12} (PIHB), folate (PIHF), omega-3 fatty acid (PIHO), and combined supplementation (PIHC) (micronutrients and omega-3 fatty acids) (Figure 1).

On 20 d of gestation, half the dams in each group were dissected and sacrificed to collect the placenta for molecular estimations. On 22 d of gestation, the remaining half of the dams was allowed to deliver normally. During lactation, both dams and their offspring were switched to a control diet and then the offspring were followed to 3 month of age, dissected and sacrificed to collect samples of liver tissue. The tissue was collected in cryovials and stored immediately at −80°C for further molecular estimations.

Induction of PIH

NG-nitro-L-arginine methylester (L-NAME) (50 mg/kg body weight/d) was administered by means of oral gavage from 14−19 d to induce hypertension during pregnancy in a rat, as previously reported by us (20).

Diet Preparation

Vitamin-free casein was used for preparing the treatment diets. Diets were prepared following AIN-93 guidelines for purified diets for laboratory rodents (22). The source of omega-3 fatty acids of the diets in this study was provided using MaxEPA capsules which contained a mixture of 180 mg of eicosapentaenoic acid (EPA) and 120 mg of docosahexaenoic acid (DHA) as previously reported by us (20). Animals (dams and offspring) were fed an *ad libitum* diet from the end of pregnancy to 3 month of age.

Observations Recorded

Organ weights. The absolute organ weights (of offspring placenta and liver) were recorded on an electronic scale (Schimadzu, minimal count of 0.001 g) and snap frozen in liquid nitrogen and stored at −80°C for taking several molecular estimations. The relative organ weight of these organs was calculated as:

Relative organ weight (%) = [(organ weight/weight of the animal)*100].

RNA isolation and cDNA synthesis. Trizol reagent (Invitrogen) was used to isolate the total RNA from the offspring placenta and liver. A Biophotometer (Eppendorf, Germany) was used to quantify total RNA. The reverse transcription (RT) of total RNA (2000 ng) to single-stranded cDNA was carried out using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) in a 20 μl reaction previously described by us (23).

Analysis of IL-6, IL-10, TNF-α, VEGF and VEGFR-1mRNA levels. The quantitative PCR (qPCR) amplification was performed using cDNA equivalent to 100 ng total RNA (Applied Biosystems 7500, USA) for the following genes: IL-6, IL-10, TNF-α, VEGF and VEGFR-1. The TaqMan Universal PCR-master mix (Applied Biosystems, USA) was used. This methodology has been reported by us (23) and others (24). The primers used for the qPCR amplification are tabulated below. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used to normalize the variation in the quality of RNA and the amount of cDNA input from the sample. The relative gene expression levels was calculated by the $2^{\Delta CT}$ method which is a standard