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The Hypothesis

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Science is clearly not a fixed, unchanging set of beliefs which, once learnt, one is expected to accept forever. Science has been and is a rapidly changing body of knowledge. It is an on-going attempt by scientists to understand and explain the living world that is why we should never look on science as a fixed set of beliefs laid down by some distant authority, which we are required to accept unquestioningly. On the contrary, one is expected and encouraged to be skeptical and critical of what one is told, or what one read. "What is the evidence of for this statement? What assumptions are implied in this explanation? Is this conclusion justified on the evidence, or would one be wiser to keep and open mind? It is on this premise that we wish to suggest a cause for preeclampsia-eclampsia. This cause has novel features which are of considerable biological interest.

The theory posited by Roberts and his colleagues in 1989 has continued to guide research related to preeclampsia-eclampsia aetiology (Roberts *et al*., 1989). Drawing on past work that associated preeclampsia with shallow trophoblast invasion and subsequent reduction in placental perfusion, they hypothesized that the ischaemic placenta released a damaging factor(s) into the maternal circulation. Although the identity of this factor was hitherto unknown, the circulating factor was hypothesized to have caused endothelial dysfunction and would lead to activation of coagulation cascade, blood pressure abnormalities and loss of fluid from intravascular space. In our opinion, this placental factor is unsatisfactory for two reasons. Firstly, we believe that the factor which causes endothelial dysfunction is a genetic material of embryonic origin and not a gene product released by the placenta. Without the genetic material, it is not clear why the disease condition will be seen in primigravidas or during first pregnancy in multiparous women who have changed partner. Secondly, the placental factor does not cause endothelial dysfunction in subsequent pregnancies if it did not cause damage in the first pregnancy except when there is multiple gestation or

molar pregnancy.

The 1980 discovery of nitric oxide as an endothelial cell-derived relaxing factor resulted in an unprecedented biomedical research of nitric oxide and established it as one of the most important cardiovascular system molecule. A reduction in endothelial cell nitric oxide levels leading to "endothelial dysfunction" has been identified by several investigators as a key pathogenic event preceding the development of hypertension. The reduction in endothelial nitric oxide in cardiovascular disease has been attributed to the action of antioxidants that either directly react with nitric oxide or uncouple its substrate enzyme. Gersch and his co-worker demonstrated in 2008 that uric acid reacts directly with nitric oxide in a rapid irreversible reaction resulting in the formation of 6-aminouracil and depletion of nitric oxide (Gersch *et al*., 2008; Khosla *et al*., 2005).

As a follow-up to this research finding, we designed a study to assess the level of uric acid in maternal circulation during normal pregnancy and we found out that uric acid level was significantly higher in pregnant women than none pregnant women with levels in the 4^{th}, 7^{th} and 8^{th} months being higher when compared to the other months of gestations. Based on this, we propose that the damaging factor released into the maternal circulation by the ischaemic placenta is uric acid produced from the cells of embryonic origin. This might explain why women with molar and multiple pregnancies develop preeclampsia-eclampsia.

Uric acid is generated in mammalian systems as an end-product of purine metabolism. Hyperuricemia may result when there is increased synthesis of purine, increased purine intake, increased turnover of nucleic acids, increased tissue breakdown or increased tissue damage. It possesses free-radical-scavenging properties (Kuzkaya *et al*., 2005; Robinson *et al*., 2004) and is the most abundant antioxidant in human plasma (Ames *et al*., 1981; Hediger, 2002). In addition, uric acid inhibits system A amino acid uptake (Bainbridge *et al*., 2009) and has

endogenous danger signaling properties (Behrens *et al*., 2012) that ultimately lead to suppression of growth rate. We wish to put forward a radically different evidence for uric acid as a cause of preeclampsia-eclampsia. This evidence is anthropometric in nature (Fig 1, 2). From figure 1, it can be seen that during intrauterine life, fetal growth rate is high from week 13 – week 18 but as the fetus enters week 19, the growth rate drops as low as 1mm/day (Mador *et al*., 2012). We assumed that during this period, the level of uric acid in maternal circulation is high reflecting its level in fetal circulation. This high uric acid level in fetal circulation most likely inhibits system A amino acid uptake and triggers endogenous signaling properties of uric acid in fetal tissue leading to suppression of growth rate in the fetus as seen during week 19. In addition to suppressing growth rate, it reduces endothelial cell nitric oxide levels leading to "endothelial dysfunction" which has been described as the key pathogenic event preceding the development of hypertension. Furthermore, suppression of growth rate around the 19th week makes the fetus to rapidly loose approximately 318 grams. We assumed that as uric acid level rises in the 4th month of life, it reacts directly with endothelial nitric oxide thereby depleting it leading to endothelial dysfunction hence the manifestation of preeclampsia-eclampsia as from 20 weeks of gestation. As fetal growth and development continues uric acid level in maternal circulation drops from higher levels at the 4th month only to peak again around the 7th and 8th months of life. Since the fetal purine which is unique is determined by the fusion of maternal and paternal gametes at fertilization, it elicits immunological response that will protect the endothelial cells of maternal vasculature in subsequent pregnancies. However, when such a woman changes partner, her first pregnancy with the new husband will form different sets of purine at fertilization which will be new when released in the maternal circulation thereby eliciting a fresh response in the mother causing endothelial dysfunction. In subsequent pregnancies, the maternal immune system would have developed defense against the uric acid produced. In multiparous women with twin pregnancy, the level of uric acid produced doubles the level of singleton pregnancies thereby

readjusting the nitric oxide depletion threshold leading to endothelial dysfunction. Similarly, in molar pregnancy the level of uric acid produced is much higher than the level in singleton and multiple pregnancies thereby giving and insight as to why preeclampsia-eclampsia occur earlier in molar pregnancy before 20 weeks. Going by Hill's criteria for causation, uric acid fulfills almost all the criteria outlined.

The novel feature of uric acid as the cause of preeclampsia-eclampsia is the manner in which its synthesis can be regulated using xanthine oxidase inhibitors during first pregnancies in order to prevent the occurrence of the disease and its ability to activate immune effectors of both the innate and adaptive immune systems thereby preventing the disease in subsequent pregnancies. Towards this end, we are planning to carry out randomized clinical trial in order to determine the usefulness of xanthine oxidase inhibitors in the prevention of preeclampsia-eclampsia as well as its use in the treatment of pre-term labour. It has not escaped our notice that depletion of nitric oxide from the uterine myometrium at term might be responsible for causing the onset of labour.