

Role of Bilirubin – a product of Heme-oxygenase system in Preeclampsia

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Abstract

Hypertension in pregnancy is a leading cause of both maternal and fetal mortality and morbidity. Endothelial dysfunction and oxidative stress play a key role in the etiopathogenesis of preeclampsia. Recently hemeoxygenase-1 enzyme has been implicated as a contributing factor to the antioxidant capabilities in several organ systems. The objective of this study to evaluate hemeoxygenase-1 product, bilirubin levels in preeclamptic women. Thirty normal pregnant women attending the antenatal clinic and thirty preeclamptic women were recruited from Obstetrics and Gynecology Department, Narayana General Hospital, Nellore. Serum bilirubin levels were significantly elevated in preeclamptic women when compared with that of normal pregnant women (pvalue < 0.000). This suggests that induction of hemeoxygenase-1 or its metabolites may be a promising therapeutic approach in the treatment of preeclampsia.

Keywords: Bilirubin, Preeclampsia, Hemeoxygenase-1

Introduction

Hypertensive disorders during pregnancy complicate 7-10% of total pregnancies, out of which 70% are preeclamptic. It increases perinatal mortality by fivefold and kills 50000 women worldwide¹. Preeclampsia is a multisystem disorder characterised by hypertension to the extent of 140/90 mm of Hg or more, proteinuria (≥ 300 mg/day) and oedema induced by pregnancy after 20th week. Without intervention, preeclampsia progresses to eclampsia.

Despite considerable research, the pathophysiology of preeclampsia remains unclear². However, oxidative stress has been attributed to be the causative factor of preeclampsia³. Heme oxygenase enzyme

typically catalyses the rate limiting step in the heme salvage pathway, converting the prooxidant heme to biliverdin, which is then rapidly converted by biliverdin reductase to bilirubin, a known antioxidant^{4,5}. Very scanty literature is available on the role of bilirubin in preeclampsia. Few studies have shown elevated serum bilirubin^{6,7} and some studies reported decreased serum bilirubin levels⁸ in preeclampsia. Therefore, the present study was undertaken to evaluate the serum bilirubin levels in subjects with preeclampsia.

Materials and methods

30 normotensive pregnant women attending antenatal clinic and 30 cases of preeclampsia

were recruited from Obstetrics and Gynecology Department, Narayana General Hospital, Nellore, for the study. After the study was approved by Institutional Ethics Committee, informed consent was taken from all study participants. Both cases and controls were between 20-30 yrs of age and were in their third trimester.

Inclusion criteria:

Non-smoker, primigravida with singleton pregnancy. Women with Preeclampsia diagnosed based on definition of American College of Obstetricians and Gynecologists (ACOG)s : 1) Systolic Blood Pressure greater than 140 mm of Hg or rise of at least 30 mm of Hg or 2) Diastolic Blood Pressure greater than 90 mm of Hg or rise of at least 15 mm of Hg (manifested on two occasions at least 6 hrs apart) and 3) Proteinuria of 300 mg or greater in 24 hrs urine collection or protein concentration of 1 gm/litre (on two occasions at least 6 hrs apart) ⁹. Subjects with normal pregnancy were normotensive and had no proteinuria.

Exclusion criteria:

Illness like severe anemia, diabetes mellitus, liver disease, renal disease, chronic hypertension and twin pregnancy were excluded.

Methods:

Under complete aseptic conditions, 5 ml of venous blood was collected and serum was separated. Bilirubin estimation (dichlorophenyl-diazonium-tetra fluoroborate (DPD) method) was done by Automated chemistry analyser [Humastar 600 (Human Gm BH Germany)] using available commercial kit.

Statistical analysis:

Data was analysed using statistical software SPSS version 20. Values are expressed as mean \pm standard error of mean (SEM). Comparison of values between cases and controls was done using student's t test. A p

value of less than 0.05 was considered statistically significant.

Table 1: Comparison of serum bilirubin levels between preeclamptic and normal pregnant women

Parameter	Preeclamptic Women (n =30) Mean \pm SEM	Normal Pregnant Women (n= 30) Mean \pm SEM	P value
Serum Bilirubin (mg/dl)	0.75 \pm 0.019	0.54 \pm 0.03	0.000*

*p value < 0.05 statistically significant, n= number of subjects

Results and discussion

Uncontrolled lipid peroxidation is an important factor in the pathogenesis of preeclampsia¹⁰.

In the present study, serum bilirubin levels were statistically significantly elevated in preeclamptic women (p value <0.00) compared to that of normotensive pregnant women, indicating an increase in heme oxygenase activity. Similar findings were noted by others^{6,7}. In a recent retrospective observational study of 50,712 pregnancies, 925 of which involved preeclampsia demonstrated that low levels of bilirubin were associated with poor maternal and infant outcomes in women diagnosed with preeclampsia, indicating that bilirubin may act as antioxidant in this condition and thus modify the disease¹¹. In contrast to our study, decreased serum bilirubin levels were observed in preeclamptic women compared to that of normotensive pregnant women by Dina M. Abo Elmatty et al⁸.

Bilirubin is the endproduct of catabolism. Bilirubin has been shown in numerous systems to function as powerful antioxidant, and moderate increases could act to decrease overall oxidative stress^{4,5}. Heme oxygenase is the rate limiting enzyme for bilirubin production. Of the three isoforms of

hemeoxygenase ,HO-1 is inducible. It is induced by heme,and other inducers like oxidative stress , metals , hypoxia and inflammatory cytokines ¹². Invitro studies indicate that HO-1 induction significantly reduces hypoxia induced oxidative stress from rodent placental villous explants, which is also recapitulated by exogenous bilirubin administration ^{13,14}. Hemoproteins are responsible for the transfer of oxygen from mother to fetus during pregnancy and heme is produced by the trophoblast , the site of exchange of substances between maternal blood and fetal circulation. Placental vascular endothelium is exposed to high concentrations of hemoglobin and free heme can undergo autooxidation to produce superoxide and hydrogen peroxide , which in turn promote reactive oxygen species(ROS) formation and damaging free radicals. Because of its direct contact with fetal blood , fetal heme is likely to be degraded in the syncytiotrophoblast by hemeoxygenase system to release bilirubin and CO in the maternal circulation., hence contributing to fetal vasodilatation of the placental villi ¹⁵.

Conclusion

We conclude that bilirubin acts as antioxidant contributing to vasoprotection in preeclampsia. Heme oxygenase system or its main metabolites, bilirubin and CO may be used as targets for developing novel drugs for the treatment of preeclampsia. Future studies both invitro and invivo are necessary to fully elucidate the safety and efficacy of agents to increase hemeoxygenase -1 or its metabolites in treating preeclampsia

Conflict of interest: None

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