

# Cord Blood Angiogenic Profile in Normotensive Pregnancies

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## ABSTRACT

Human placenta undergoes both angiogenesis and vasculogenesis during fetal development. An imbalance in proangiogenic [placental growth factor (PIGF) and vascular endothelial growth factor] and antiangiogenic factors [soluble fms like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng)] has been reported to have a role in pathophysiology of preeclampsia. Hence the present study was designed to analyze heme oxygenase-1 and endoglin levels in maternal and cord blood of normotensive women. The study comprised of twenty five normotensive pregnant women immediately after delivery. Study samples were drawn (maternal venous blood and umbilical cord blood) and heme oxygenase-1 (HO-1) and endoglin levels were analyzed by competitive enzyme linked immunosorbent assay. Heme oxygenase-1 levels were elevated in maternal blood as compared to cord blood of normotensive pregnant women. Also, serum blood endoglin levels were higher in maternal blood compared to cord blood of normotensive pregnant women. These findings indicate that there is angiogenic balance during normotensive pregnancy and dysbalance might occur during pathological pregnancy. These markers of angiogenic balance may serve as diagnostic marker and may help in explaining future risk of cardiovascular disease in these women.

**KEYWORDS:** Vasculogenesis; Proangiogenic and antiangiogenic factors; Pregnancy

## INTRODUCTION

During pregnancy, there is coordinated formation of new blood vessels (termed as angiogenesis). Vascular growth factors, vascular endothelial growth factor and placental growth factor, promote remodelling of the maternofetal interface. Placental and maternal vasculatures are major sources of reactive oxygen and nitrogen species that alter vascular function in preeclampsia. Placental hypoxia and maternal vascular dysfunction occur by linking of placental syncytiotrophoblast basement membranes shedding with placental angiogenic factors [soluble flt1 and endoglin]. Endoglin binds vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) in the maternal circulation [1,2].

Angiogenesis is critical in establishing a functional placenta during pregnancy. During preeclampsia, insufficient maternal spiral artery remodelling occurs due to defective endovascular invasion by cytotrophoblasts and impaired vasculogenesis [1,2]. Normally, there is a balance between proangiogenic and antiangiogenic factors in the developing placenta. Deregulation of angiogenesis in placenta and maternal circulation results in placental insufficiency and its further consequences

such as fetal growth restriction and maternal preeclampsia. In the event of any imbalance in proangiogenic [placental growth factor (PIGF) and vascular endothelial growth factor] and antiangiogenic factors [soluble fms like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng)], angiogenesis will be altered and preeclampsia may ensue [1-3].

The main determinants of vasodilatory and angiogenic reserve are angiogenic growth factors (GFs) resulting in a favorable anti- to pro-angiogenic balance in pregnant women. HO-1 maintains uterine quiescence and overexpression of HO-1 has been reported to decrease the production of sFlt-1 and sEng. The concept that HO-1 acts as a negative regulator of sFlt-1 and sEng release also indicates that this cascade offers vascular protection against pregnancy induced oxidative stress and exacerbated inflammation. It has already been reported that protein levels of HO-1 in preeclamptic placenta are decreased. The angiogenic balance in preeclampsia might be influenced by HO/CO-system [3,4]. The present study explored a range of HO-1 and endoglin as a potential marker of angiogenic balance and their association with clinically relevant pregnancy outcomes.

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Limited information is available regarding pathophysiological events of fetoplacental side during gestation and placenta terminates self-role as the fetomaternal mediator soon after the delivery. Altered antiangiogenic signaling and unfavorable proinflammatory cytokines production by placenta have been implicated in pathophysiology of preeclampsia [3,4].

This article aimed to explore association of maternal characteristics and fetal outcome with angiogenic factors of normotensive pregnant women.

## MATERIALS AND METHODS

The study was carried out in the Departments of Biochemistry and Obstetrics and Gynaecology, Pt. B.D. Sharma, PGIMS, Rohtak. Serum heme oxygenase-1(HO-1), endoglin and lipid profile were analyzed in maternal and cord blood in normotensive pregnant women. An informed consent was taken from all the patients and the research protocol was approved by the Institutional Review Board. Women with history of smoking, any metabolic disorder before or during pregnancy or presence of high risk factors like anemia, heart disease, diabetes, renal disease, history of any vitamin supplement were excluded from the study. All clinical data were collected after the initial preeclampsia evaluation including age, height, weight, smoking status, gestational age, clinical findings, BP, and the results of laboratory tests were included. Also, pregnancy outcomes including complications and delivery characteristics were recorded.

Twenty five pregnant women with singleton pregnancies who remained normotensive (defined as BP<140/90) throughout the pregnancy were selected and study samples (maternal venous blood and umbilical cord blood) were drawn immediately after delivery. Heme oxygenase-1 was analyzed by competitive enzyme linked immunosorbent assay (QAYEE-BIO) [5]. RayBio® Human Endoglin (CD105) ELISA (Enzyme-Linked Immunosorbent Assay) kit was used for the quantitative measurement of human Endoglin in blood.6 The intra-assay and inter-assay coefficients of variation were <10% and <12%. Lipid profile was analyzed enzymatically. All the analyses were performed by using the statistical package (SPSS 20). The values were compared with non-pregnant control values.

## RESULTS

The mean age of normotensive women was  $24.16 \pm 1.86$  years and mean gestational age at time of delivery was  $37.52 \pm 0.59$  weeks. Gestational age of mothers ranged from 34-41 weeks at time of delivery. Majority of the babies had birth weight between 2.1-2.5 kg and mean birth weight was  $2.34 \pm 0.19$  kg. Majority had vaginal delivery in normotensive mothers. Mean birth weight in normotensive women was  $2.34 \pm 0.19$  kg. Majority of

the babies were born with good Apgar score. Majority of mother had vaginal delivery 84% and assisted deliveries (vacuum/ forceps) in 2%.

The maternal heme oxygenase-1 (HO-1) were higher as compared with cord blood ( $277.20 \pm 32.24$  ng/dl and  $276.60 \pm 31.62$ ng/dl respectively). The maternal endoglin were higher as compared with cord blood ( $6.64 \pm 0.34$  ng/dl and  $6.60 \pm 0.28$ ng/dl respectively).

Maternal HO-1 were higher in mothers with female babies as compared to male babies ( $285.30 \pm 28.40$  vs.  $268.41 \pm 35.00$  ng/ml,  $p>0.05$ ). Cord blood HO1 were higher in female babies as compared to male babies ( $279.23 \pm 30.95$  ng/ml vs.  $273.75 \pm 33.45$  ng/ml,  $p>0.05$ ). Cord blood heme oxygenase 1 was 99.78% of maternal levels and cord blood endoglin levels were 99.39% of maternal levels.

There was a negative correlation between maternal heme oxygenase 1 and maternal endoglin in normotensive pregnant women ( $r=-0.039$ ,  $p>0.05$ ). Positive correlation was observed between cord blood HO1 and cord blood endoglin in normotensive pregnant women ( $r=0.159$ ,  $p>0.05$ ).

Negative correlation was observed between cord blood HO1 with gestational age ( $r=-0.209$ ,  $p>0.05$ ). Negative correlation was observed between cord blood endoglin and gestational age ( $r=-0.167$ ,  $p>0.05$ ).

Positive correlation was observed between maternal HO1 levels with birth weight ( $r=0.098$ ,  $p>0.05$ ). Positive correlation was observed between cord blood HO1 and birth weight ( $r=0.186$ ,  $p>0.05$ ). Significant positive correlation was observed between cord blood endoglin and birth weight ( $r=0.404$ ,  $p<0.05$ ).

In the present study, maternal HO-1 showed positive correlation with MBP (mean blood pressure) in normotensive women, however, it was not statistically significant ( $r=0.116$ ,  $p>0.05$ ). In the present study, maternal HO1 showed a positive correlation with systolic blood pressure in normotensive women ( $r=0.169$ ,  $p>0.05$ ). Maternal HO1 showed a positive correlation with diastolic blood pressure in normotensive women, however, it was not statistically significant ( $r=0.396$ ,  $p>0.05$ ).

Maternal HO 1 levels in the present study showed a positive correlation with Hb in normotensive women but it was not statistically significant ( $r=0.166$ ,  $p>0.05$ ). In the present study, serum bilirubin levels were  $0.70 \pm 0.12$  mg/dl in normotensive pregnant women. Serum bilirubin levels negatively correlated with HO 1 levels in normotensive pregnant women ( $r= -0.292$ ,  $p>0.05$ ).

In the present study, Maternal LDL levels were negatively correlated in normotensive women ( $r=-0.111$ ,  $p>0.05$ ). Cord blood LDL levels were positively correlated in normotensive women ( $r=0.142$ ,  $p>0.05$ ).

## DISCUSSION

Coordinated formation of new blood vessels during pregnancy occurs under influence of vascular growth factors, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) that promote remodelling of the maternofetal interface. Soluble flt1 and endoglin are placental angiogenic factors and endoglin binds vascular endothelial growth factor and placental growth factor in the maternal circulation [4-6]. For normal placental function, fine balance between angiogenic and anti angiogenic signaling is very important and any dysregulation in expression of key angiogenic factors may contribute to a number of obstetric complications including preeclampsia.

The maternal heme oxygenase-1 was higher as compared with cord blood and maternal endoglin were higher as compared with cord blood [Table 1]. Padmini et al. reported decreased expression of HO-2 in cord blood RBC of preeclamptic subjects compared to normotensive subjects [7]. Cord blood heme oxygenase 1 was 99.78% of maternal levels and cord blood endoglin levels were 99.39% of maternal levels. To the best of our knowledge no study is available regarding cord blood HO-1 in normal pregnancy.

	HO-1 (ng/dl)			Endoglin (ng/dl)		
	Maternal	Cord blood	%	Maternal	Cord blood	%
Normotensi ve pregnant	277.20 ± 32.24	276.20 ± 31.62	99.78	6.64 ± 0.30	6.60 ± 0.28	99.39

\*p<0.05 as compared to group-I  
\*\*p<0.001 as compared to group-I

**Table 1:** Maternal and cord blood heme oxygenase-1 and endoglin levels in both groups [(ng/ml, mean + SD)].

Negative correlations were observed between cord blood HO1 and endoglin with gestational age ( $r=-0.209$ ;  $r=-0.167$ ,  $p>0.05$ ). Significant positive correlation was observed between cord blood endoglin and birth weight ( $r=0.404$ ,  $p<0.05$ ). Negative correlation was observed between gestational age and both cord blood HO-1 and endoglin levels. The effect of gestational age at delivery on the differences in level is not known and further studies are required in this aspect to explore whether primary pathology is uteroplacental response to fetal placental vasculature or uterine circulation constraints on placenta and fetus.

Low birth weight have been reported to predict predisposition to atherosclerotic cardiovascular diseases in later life [8,9]. Also, reduced fetal growth carries a risk of hypertension and ischemic heart disease in later years [10-12]. A significant positive correlation was observed between cord blood endoglin and birth weight ( $r=0.404$ ,  $p<0.05$ ). Endoglin and soluble endoglin expression have

been reported to be significantly increased in placentas of IUGR singletons compared to controls [13]. Laskowska et al. reported increased endoglin levels in women with normotensive pregnancy complicated by IUGR and in preeclampsia with and without IUGR [14]. However, levels of sEng at term pregnancy are not known. On reviewing the literature, no reports are available where HO-1 and birth weight have been compared.

A positive correlation was found between maternal HO-1 and MAP normotensives suggesting that HO-1 plays a role in maternal vascular tone regulation and HO-1 deficiency can induce elevations in maternal diastolic blood pressure. Zhao et al. reported association of diastolic blood pressure with HO-1 deficiency [12], suggesting that HO-1 plays a role in regulation of maternal vascular tone. Thus agents that increase HO expression and reduce release of antiangiogenic factors may be beneficial as therapeutic agents in preeclampsia. George et al. have demonstrated that pharmacological inhibition of HO-1 in late pregnancy caused a significant increase in MBP and it was associated with decrease VEGF production by the placenta and elevated placental NADPH oxidase, major sources of superoxide [15].

In the present study, both maternal heme oxygenase 1 and endoglin levels were higher as compared to cord blood counterparts in normotensive pregnant women.

Antiangiogenic profile defined in terms of HO-1/Eng ratio was 41.76 in normotensive pregnant women. In normotensive HO/Eng+ IGF were lower (0.76) in normotensive pregnant and 16 (64%) subject had HO-1/Eng also less than 41.76. For a successful pregnancy, there needs to be a balance of pro- and antiangiogenic proteins. If the normal physiologic increase in antiangiogenic factors towards the end of pregnancy occurs too soon and/or if there is an excess production of the antiangiogenic proteins, preeclampsia may result.

Maternal HO1 were higher in mothers with female babies as compared to male babies. Cord blood HO1 was higher in female babies as compared to male babies. Gender-based differences in the incidence of hypertensive and coronary artery disease, the development of atherosclerosis are known and they occur as a result of differences in hormones, lipid profile, myocardial, endothelial, and vascular performance between male and female gender and aging [16].

Relatively little is known about the effect of sex hormones on HO activity. Demonstration of gender based changes in the present study supports the idea of active contribution of placenta to metabolism of maternal during pregnancy.

The findings of present study indicate that a balance of pro- and antiangiogenic proteins is necessary for a successful pregnancy and any increase in antiangiogenic factors may result in preeclampsia.

## CONCLUSION

Demonstration of gender based changes in the present study supports the idea of active contribution of placenta to metabolism of maternal during pregnancy. The findings of present study indicate that a balance of pro- and antiangiogenic proteins is necessary for a successful pregnancy and any increase in antiangiogenic factors may result in preeclampsia. These markers of angiogenic balance may serve as diagnostic marker and may help in explaining future risk of cardiovascular disease in these women.

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## Conflict of Interest

None.

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## REFERENCES

1. Demir R, Kaufmann P, Castellucci M, Erben T, Kotowski A. Fetal vasculogenesis and angiogenesis in human placental villi. *Acta Anat (Basel)* 1989;136:190–203.
2. Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, et al. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. *J Clin Invest* 2004;114:744–54.
3. Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Cuxall H, Chudasama K. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase. *Circulation* 2007;115:1789–97.
4. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. *Annu Rev Med* 2008;59:61–78.
5. Gilbert JS, Verzwylt J, Colson D, Arany M, Karumanchi SA, Granger JP. Recombinant vascular endothelial growth factor 121 infusion lowers blood pressure and improves renal function in rats with placental ischemia-induced hypertension. *Hypertension* 2010;55:380–5.
6. Warrington JP, George EM, Palei AC, Spradley FT, Granger JP. Recent advances in the understanding of the pathophysiology of preeclampsia. *Hypertension* 2013;62:666–73.
7. Padmini E, Lavanya D. HIF1 $\alpha$ , TNF $\alpha$  and HO-1 modulation in placental explants during Preeclampsia. *Int J Res chem Environ* 2014;4:85–92.
8. Barker DJ. Intrauterine programmes of coronary heart disease and stroke. *Acta Paediatr* 1997;423:178–82.
9. Haladay A. Early risk factors for cardiovascular disease. *BNF Nutr Bull* 1990;15:6–7.
10. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero blood pressure in childhood and adult life and mortality from cardiovascular disease. *BMJ* 1989;298:564–7.
11. Barker DJP, Hunter PD, Osmonde, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;9:577–80.
12. Zhao H, Wong RJ, Kalish FS, Nayak NR, Stevenson DK. Effect of heme oxygenase-1 deficiency on placental development. *Placenta* 2009;30:861–8.
13. Yinon Y, Nevo O, Xu J, Many A, Rolfo A, Todros T. Severe intrauterine growth restriction pregnancies have increased placental endoglin levels. *Am J Pathol* 2008;172:77–85.
14. Laskowska M, Laskowska K, Oleszczuk J. Endoglin in pregnancy complicated by fetal intrauterine growth restriction in normotensive and preeclamptic pregnant women: a comparison between preeclamptic patients with appropriate-for-gestational-age weight infants and healthy pregnant women. *J Matern Fetal Neonatal Med* 2012;25:806–11.
15. George EM, Colson D, Dixon J, Palei AC, Granger JP. Heme oxygenase-1 attenuates hypoxia-induced sFlt-1 and oxidative stress in placental villi through its metabolic products CO and bilirubin. *Int J Hypertens* 2012.
16. Szentpéteri I, Rab A, Kornya L, Kovács P, Brubel B, Joó JG. Placental gene expression patterns of endoglin (CD105) in intrauterine growth restriction. *Mat Fe Neo Med* 2014;27:350–4.