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IS PLACENTAL INFLAMMATION A RISK FACTOR FOR INTRAUTERINE GROWTH RESTRICTION?

Krishna Rao Gurugubelli[1], Vishnu Bhat Ballambattu[2]*, Zacheriah Bobby[3], Bhawana Badhe[4], Dasari Papa[5]

[1,2]Department of Neonatology, Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER), Puducherry-605006, India.
[3]Zachariah Bobby, Senior Professor, Biochemistry, JIPMER, Puducherry
[4]Bhawana Badhe, Senior Professor, Biochemistry, JIPMER, Puducherry.
[5]Dasari Papa, Professor & Head, Obstetrics & Gynaecology, JIPMER, Puducherry.

***Corresponding Author: Dr. Vishnu Bhat Ballambattu**
Department of Neonatology, Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER), Puducherry-605006, India.

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ABSTRACT

Background: Intrauterine growth restriction (IUGR) is a pregnancy associated disease manifested by decreased growth rate of fetus than the normal genetic potential. IUGR represents a human model of chronic fetal hypoxia. Hypoxia induced ischemic tissue injury leads to inflammation and production of pro inflammatory cytokines. **Objectives:** To investigate the relation of inflammatory mediators in cord blood with intrauterine growth restriction. **Methods:** This cohort study was conducted in the Neonatology Department, JIPMER, a tertiary care hospital in south India from August 2016 to July 2017. Thirty seven (37) IUGR and thirty eight (38) AGA neonates were recruited. Umbilical cord blood samples were collected at birth and anthropometric measurements were recorded. Plasma was separated from the cord blood samples and TNF-α, IL-6 were measured using high sensitivity enzyme linked immunosorbent assay (ELISA) kits. Levels of inflammatory mediators were correlated with anthropometric measurements of babies using statistical tests. **Results:** IUGR neonates had lower birth weight (BW), birth length (BL) and head circumference (HC). Their plasma levels of IL-6 were significantly higher in the unadjusted model. The results of stepwise multivariate linear regression analysis showed that after adjusting for sex, gestational age, birth weight, birth length and head circumference, there was no significant difference in IL-6 levels between IUGR and AGA babies (p=0.09). TNF-α was also not significantly associated with any of the variables (F=0.8). **Conclusion:** Our study demonstrated that, in adjusted model both IUGR and AGA had comparable IL-6 and TNF-α level suggesting that placental inflammation may not be a risk factor IUGR.

KEYWORDS: Intrauterine growth restriction; Inflammatory mediators; Placenta; Cord blood plasma.

INTRODUCTION

Intrauterine growth restriction (IUGR) is a pregnancy related disease manifested by decrased growth rate of foetus than the normal genetic growth potential at particular gestational age. Majority of IUGR babies are small for gestational age in which birth weight is less than the 10th percentile for a particular gestational age.[1-3] Fetal growth is a complex process and is governed by several factors originating from mother, placenta or foetus. In early foetal life significant factor for growth is foetal genome but in later stages of pregnancy it is regulated by interaction between multiple factors, such as nutritional, hormonal, environmental and genetic factors.[4] IUGR has many possible causes, a common cause being placental dysfunction and is believed to be the chief pathogenic mechanism responsible for inadequate nutrient supply from mother to foetus.[5] IUGR represents a human model of chronic foetal hypoxia.[6,7] Hypoxia induced ischemic tissue injury constitutes features of inflammation including production of pro - inflammatory cytokines.[8] Several cytokines are involved in the proinflammatory and anti- inflammatory effects. Among them interleukin-6 (IL-6) is a multifunctional cytokine secreted by macrophases and T-cells and is an important mediator of fever and acute phase response.[9-11] Tumor necrosis factor-α (TNF-α) is mainly produced by macrophases and also produced by other cell types, such as NK cells, CD4+ Th1 helper cells and placenta.[12]

Anthropometric measurements like birth weight, birth length and head circumference have been used for predicting the complications of IUGR.[13] However these measurements are not sensitive enough to detect

abnormalities of IUGR. Therefore we investigated the relation of cord plasma levels of inflammatory mediators with BW, BL, HC in IUGR and AGA (Appropriate for gestational age) neonates.

METHODS

This study was approved (No. JIP/IEC/2015/23/797) by Institute Ethics committee (Reg.No: ECR/342/Inst/PY/2013) of JIPMER hospital. Written informed consent was obtained from parents of the neonates. Five ml of cord blood was collected from 37 IUGR and 38 AGA neonates admitted to JIPMER, a tertiary care hospital in south India from IUGR neonates were diagnosed as babies with birth weight less than 10th percentile for the gestational age and antenatal ultrasound showing either absent or reversed end diastolic flow velocities on at least 50% of the Doppler waveforms from the umbilical artery on at least one occasion during pregnancy. Neonates who had birth weight between 10th-90th percentile and normal antenatal ultrasound was considered as a control group.We excluded infants with congenital malformations, maternal history of infections and inflammations. Fig.1 represents the design of the study.

Cord blood was collected from all cases and controls. Plasma was separated from the blood samples and TNF-α, IL-6 were measured by High sensitivity Enzyme Linked Immunosorbent Assay (ELISA) kits using the manufactuer' s directions (Bioassay technology laboratory cat.No E0082Hu and cat.No E0090Hu respectively). Birth weight (BW) and birth length (BL)were obtained from each IUGR and AGA babies immediately after birth using standard techniques. Measurement of HC was done on 2nd day to allow for resolution of moulding.

Statistical analysis

The significant mean difference between IUGR and AGA was determined by student' s t-test. A stepwise multivariate linear regression analysis was performed to find out the independent relation between inflammatory markers(IL6,TNF) and groups(IUGR and AGA). Data was expressed as mean ± standard deviation. P value <0.05 was considered as significant.

RESULTS

IUGR neonates had significantly lower GA, BL, BW and HC than AGA neonates ($P < 0.001$). Plasma levels of IL-6 was significantly greater in IUGR neonates than in AGA neonates ($P < 0.05$). There was no statistical significant difference in TNF-α values between IUGR and AGA groups (Table.1). Stepwise multivariate linear regression analysis showed that after adjusting for sex, gestational age, birth weight, birth length and head circumference, IL-6 & TNF-α levels between the groups (IUGR and AGA) were not significant (Table.2).

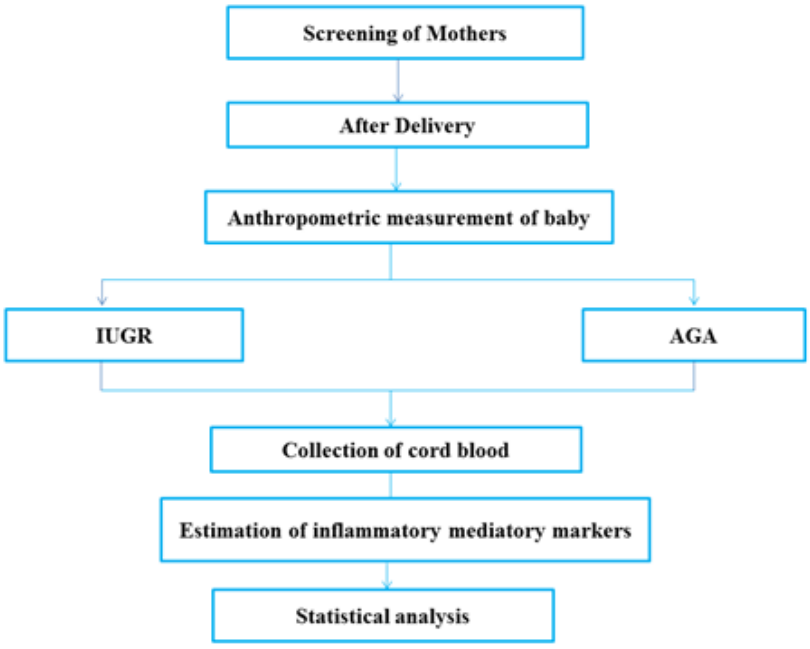

Fig. 1: Design of the study.

Table 1: Clinical characteristics and inflammatory markers of IUGR and AGA neonates.

Variable	IUGR (n=37)	AGA (n=38)	P-Value
Gender (Male/Female)	15/22	24/14	
Gestational age (weeks)	35.68 ± 3.2	39.5 ± 1.2	<0.001
Birth length (cm)	40.37 ± 2.81	48.67 ± 1.63	<0.001
Birth weight (gm)	1646.08 ± 415.43	3068.56 ± 328.27	<0.001
Head circumference (cm)	27.18 ± 2.02	34.02 ± 1.38	<0.001
IL-6 (ng/l)	65.61 ± 15.80	58.89 ± 7.51	0.020
TNF-α (ng/l)	5.23 ± 1.39	5.20 ± 1.16	0.910

IUGR- Intrauterine growth restriction; AGA- Appropriate-for-gestational- age; IL-6- Interleukin- 6; TNF-α- Tumor necrosis factor-α.
*Values are mean ± standard deviation.

Table 2: Multivariate linear regression analysis of IL-6 and TNF- α of neonates.

	Variables	Coefficient	t	p
IL-6(ng/l)	Constant	127.45	3.40	0.001
	Gestational age (Weeks)	-1.25	-1.53	0.130
	Group (IUGR & AGA)	-10.03	-1.71	0.09
	Birth weight (gm)	-0.007	-1.48	0.14
	Birth length (cm)	0.95	0.78	0.43
	Head circumference (cm)	-1.09	-0.07	0.45
TNF-α(ng/l)	Constant	5.51	1.42	0.16
	Gestational age (Weeks)	0.06	0.82	0.41
	Group (IUGR & AGA)	0.06	0.82	0.41
	Birth weight (gm)	0.00	0.18	0.85
	Birth length (cm)	-0.04	-0.38	0.70
	Head circumference (cm)	-0.02	-0.17	0.86

DISCUSSION

The growth rate of a fetus is a complex process and numerous cytokines have important role in normal placental and fetal growth.[14] Hypoxia plays a prominent role in IUGR and it has been documented in IUGR fetuses by cordocentesis[15]. Hypoxia is a well-known factor for stimulating the inflammatory response, including production of inflammatory cytokines.[8] It is possible that IUGR associated hypoxia in itself is a drive behind inflammatory response in IUGR neonates. IL-6 and other cytokines are crucial components of immune response and thus participate in the immunological aspects of the pathophysiology of IUGR. Pro-inflammatory cytokines seem to be involved in cellular events that establish and maintain pregnancy [16]. However their role has not yet been well defined in relation with fetal growth restriction. Usually the cord blood inflammatiory markers reflects the placental inflammation. This study investigated the relation of cord blood plasma levels of IL-6, TNF-α with BW, BL and HC in IUGR and AGA neonates. Understanding how various cytokines affect the disease may help us to understand the diseases process and develop intervention strategy for IUGR.**.** In our study**,** cord plasma levels of IL-6 were significantly higher in IUGR neonates compared to AGA neonates. However there was no significant difference in TNF-α levels between IUGR and AGA neonates. But after adjusting for sex, gestational age, birth weight, birth length, both groups (IUGR & AGA) should similar IL-6 and TNF- α levels.

A study conducted by Lausten-Thomsen et al reported elevated cord blood concentrations of IL-6 in IUGR fetus.[16] The IL-6 and TNF-α levels were elevated in pregnancy complications like pre-eclampsia. Afshari et al reported that IL-6 levels were significantly elevated in preeclampsia, but there was no significant difference in TNF-α levels.[17] Yuan Li et al also reported that serum levels of IL-6 and TNF-α were elevated significantly in pre-eclampsia.[18] Tosun et al reported that alterations in umbilical serum levels of IL-6 and TNF- α may also play a role in preeclampsia complicated with IUGR.[19] The results of these studies have not always been constient.

Our findings are similar to a previous study, in which non-significant elevation of TNF-α[20] and IL-6[21] in IUGR fetus were observed. In addition to that, IL-6 cord serum concentrations had no significant relation with birth weight and birth length.[21] TNF-α did not correlate with any of the variables.

Although the TNF-α and IL-6 were considered inflammatory cytokines, the reason for no significant change in the concentration of TNF-α and IL-6 is not clear. A possible explanation is that placental inflammation is not a cause for IUGR. We did not estimate the cytokine levels of maternal serum.

CONCLUSION

Our study demonstrated that, both IUGR and AGA groups had no significant difference in levels of IL-6 and

TNF- α indicating that placental inflammation is not a risk factor for IUGR.

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Conflict of Interest
Authors have no conflicts of interest to declare.

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