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Intrauterine Growth Restriction in Term Women with Histologic Chorioamnionitis*

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Abstract: The present research aims at studying the association of histologic chorioamnionitis (HCA) with intrauterine growth restriction (IUGR). Women at labor were recruited for this study from Niloufer Hospital, Hyderabad, India. These women were screened for HCA and Bacterial Vaginosis (BV). IL8 and TNFα concentration were measured in the Chorioamnion membranes collected from all these women. New born anthropometry were collected and using Lubchenco *et al*. standard, percentile weight for gestation age was assessed, sexes combined. Nearly twenty nine percent of women had histologic chorioamnionitis and these women had higher proportion of babies with less than 10th percentile birth weight, crown heel length and head circumference. Compared to women with asymmetrical growth retarded babies, women with symmetrically growth-retarded babies were associated with higher proportion of histologic chorioamnionitis, however bacterial vaginosis was not associated with either HCA or birth outcome. Women with histologic chorioamnionitis had significantly higher concentration of IL8 while women with BV had no association with cytokine secretion. The present study shows that intrauterine inflammation; a strong predictor of intrauterine infection is associated with fetal growth, while bacterial vaginosis-a lower genital tract infection is not associated. However, the association is not robust, which could be due to bias towards selecting a population that belongs to low socio economic status and thus high prevalence of chronic low-grade infection resulting in higher prevalence of inflammation in most of the women in the study. More controlled studies are required to delineate the role of infection/inflammation on intrauterine growth.

Key words: Histologic chorioamnionitis, inflammatory response, intrauterine growth restriction

INTRODUCTION

In the last few years there has been increasing emphasis on maternal infections during pregnancy, as modifiable risk factor for intra uterine growth restriction (IUGR) and preterm delivery (Gibbs, 2001; Goldenberg and Rouse, 1998; Saini *et al*., 2003). In addition to the well described transplacentally acquired syphilis, rubella, cytomegalovirus and toxoplasmosis, abnormal vaginal flora that includes *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis* and a host of anaerobic bacteria

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are implicated in poor birth outcome (Cauci *et al*., 1998). Of these infections Bacterial Vaginosis (BV) is the most commonly prevalent, but neither diagnosed nor treated routinely in India.

Intrauterine infections are often chronic and usually asymptomatic. Most women, who are later diagnosed by histologic chorioamnionitis (HCA) or culture, have no symptoms such as fever, abdominal pain, peripheral blood leukocytosis or fetal tachycardia (Gibbs, 2001; Goldenberg and Rouse, 1998; Saini *et al*., 2003; Cauci *et al*., 1998).

Recent evidence suggests that intrauterine infections may occur quite early in pregnancy and remain undetected for months (Goldenberg *et al*., 2000). Bacterial infections can occur in the maternal tissues and the fetal membranes Infection of the chorioamnion as evidenced by histologic findings or culture is known as chorioamnionitis. Histologic chorioamnionitis has been shown to be associated with preterm delivery (PTD) (Goldenberg *et al*., 2000). In term infants there is one report relating histological chorioamnionitis to IUGR (Williams *et al*., 2000).

The effect of inflammatory response in the gestational tissue (chorioamnion membranes) that may have adverse effects on fetal growth has not been explored, except a study that showed an association of IL8 and TNF α with IUGR in malaria-infected women (Smulian *et al*., 2003), while another study showed increased TNF α in women with IUGR and placental insufficiency (Bartha *et al*., 2003).

The present study used histologic chorioamnionitis IL8 and TNFa as markers of chorioamnion membrane infection and attempted to associate this with newborn anthropometry.

MATERIALS AND METHODS

Sample Selection

Recruitment of subjects, data and sample collection were carried out during December 2005 to April 2007, at Niloufer Hospital for Women and Child Health. The experiment was undertaken with the understanding and appropriate informed consent of each participant. Procedures followed are in accordance with the ethical standards laid down by ICMR's ethical guidelines for biomedical research on human subjects.

Women who were less than 16 years of age, had taken antibiotics during the previous 2 weeks, had a fetus with a known congenital malformation, had a cervical cerclage and women with PIH, diabetes, abruption placenta, placenta previa and twins were excluded from the study.

Placentas were collected from women who had uncomplicated vaginal delivery after spontaneous labor and processed within 60 min of delivery. Two sections of 2 cm^2 chorioamnion membranes were cut a few centimeter away from the ruptured site, washed briefly in cold phosphate buffered saline to remove excessive amount of blood and one section was fixed in 10% formaldehyde, which was used for scoring inflammatory cells and another section was transported in phosphate buffer saline for estimating chorioamnion membrane cytokines. The maternal and neonatal clinical records were assessed for evidence of clinical intrauterine infection as indicated by maternal temperature >37.8°C, maternal tachycardia (pulse rate >100 min^{-1}), fetal tachycardia (>160 beats min^{-1}), uterine tenderness, foul smelling liquor and neonatal sepsis.

Screening for Genital Tract Infections

All the women in the study were screened for intrauterine infections at labor using markers such as bacterial vaginosis, histologic evaluation of inflammatory cells in chorioamnion membranes and IL8 concentration in chorioamnion membranes. Bacterial vaginosis was screened by grams stain using Nugents scoring method. For histologic evaluation of chorioamnionitis, the membranes fixed in 10% neutral buffered formalin, were embedded in paraffin and sections of 5 μ thickness were stained with Haematoxylin and Eosin. A diagnosis of chorioaminionitis was made if 10 polymorphonuclear leukocytes were present per HPF in 10 nonadjacent fields.

Cytokine Assay

Lysis buffer was added to the section in PBS at 4°C, homogenized for 15 sec on ice, followed by further homogenization with a sonicator for 30 sec. One milliliter aliquot of the homogenate was taken and centrifuged for 7 min at 10,000 g; the supernatants were removed and stored frozen at -70°C until analysis. A small aliquot of each supernatant was taken and diluted 20 fold in 0.1 N sodium hydroxide for protein assay by modified (Lowry *et al*., 1951).

IL8 and TNF α were measured by sandwich ELISA as described previously (Bhaskaram *et al*., 2003). The sensitivity of the TNF α was 10 pg mL^{-1} and that of the IL8 assay was 6 pg mL^{-1}. The interassay and intrassay coefficients of variation were <10% (9.0 and 8.6% for TNF α and 7.6 and 8.0% for IL8, respectively). All ELISAs were calibrated against recombinant human cytokine standards.

Using Lubchenco *et al*. (1963), standard percentile weight for gestation age was assessed, sexes combined. The SGA/IUGR category consisted of infants below tenth percentile of weight for gestational age. Infants delivered before 37 weeks were considered to have PTD. Newborns were also classified into symmetrical and asymmetrical growth retarded taking birth weight, crown heel length and head circumference into consideration. Those with birth weight below 10th percentile of standard and with normal crown heel length and head circumference were classified into asymmetrical and those with birth weight, crown heel length and head circumference below 10th percentile of standard were classified as symmetrical growth retarded.

RESULTS

Chorioamnion membranes and new born anthropometry were collected from 73 women with term small-for gestational age date (SGA), suggesting IUGR, 59 women with PTD and 75 term appropriate for gestational age (AGA). The mean age of mothers were 23.68±0.382, 21.00±0.276, 22.40±0.442 for those with term AGA, term IUGR and PTD respectively. The mean gestational age in months were 39.4±0.111 and 39.04±0.133 for term AGA and term IUGR and 33.0±0.400 for PTD women.

Maternal and fetal characteristics are presented in Table 1 as per birth weight and gestational age. Postnatal weight (PNW) of mothers with term IUGR babies and PTD was significantly lower compared to term AGA, while height of the mothers was significantly lower in those with IUGR babies.

The birth weight, crown heel length and head circumferences were significantly lower in term IUGR and PTD compared to term AGA. Similarly, the abdominal circumference and body fat percent were significantly lower compared to term AGA (Table 1).

Of the total women in the study 28.8% had histologic chorioamnionitis. Of the term AGA, 25% had histologic chorioamnionitis, compared to 31 and 30% in IUGR and PTD. When women with

Table 1: Maternal and new born characteristics

Anthropometry	Term AGA (75)	Term IUGR (73)	PTD (59)
Maternal			
Height (cm)	152.70±0.588 (68)	150.80±0.596* (64)	152.10±0.828 (57)
Postnatal weight (kg)	48.00±0.64 (68)	45.30±0.68** (65)	45.90±0.96* (57)
Body mass index	20.60±0.25 (68)	19.90±0.27 (64)	19.80±0.41 (57)
New born			
Body weight (kg)	$2.80±0.030^{a}$ (75)	$2.26±0.026^{b}$ (73)	$1.78±0.049^{c}$ (59)
Length (cm)	$47.80±0.257^{a}$ (68)	$45.90±0.232^{b}$ (69)	$43.30±0.453^{c}$ (49)
Head circumference (cm)	$32.80±0.144^{a}$ (68)	$31.70±0.134^{b}$ (69)	$30.60±0.320^{c}$ (49)
Ponderal index	$2.60±0.144^{a}$ (68)	$2.30±0.034^{b}$ (69)	$2.20±0.045^{c}$ (49)
Abdominal circumference (cm)	$27.90±0.23^{a}$ (72)	$26.00±0.20^{b}$ (68)	$24.20±0.32^{c}$ (48)
Fat (%)	11.95±0.42***	9.25±0.46	7.80±0.71

*p<0.05 compared to FTAGA; **p<0.01 compared to FTAGA; ***p<0.0001 compared to other groups; p<0.001- Post Hoc Test; ^{a,b,c}Numbers in parentheses indicates number of subjects

Table 2: Chorioamnionitis in relation to new born anthropometry and cytokines

Chorioamnionitis	BW	Length (cm)	HC (cm)	IL8 pg g^{-1} protein	TNF α pg g^{-1} protein
Women without chorioamnionitis (128)	2.57±0.05	46.2±0.24	32.2±1.35	148.9±240.05	69.6±18.58
Women with chorioamnionitis (52)	2.43±0.09	45.0±0.50*	31.7±1.76$^{\Delta}$	2824.5±692.4*	77.0±16.08

*p<0.02, $^{\Delta}$p<0.09 trend, Numbers in parentheses indicates number of subjects

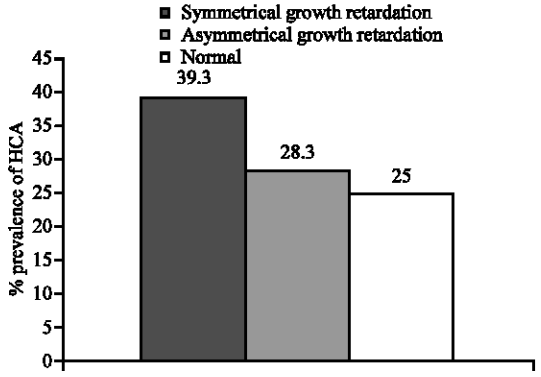

Fig. 1: Growth retardation and HCA

IUGR babies were divided into symmetrical and asymmetrical growth retarded babies, women with symmetrically growth-retarded babies were associated with higher proportion of histologic chorioamnionitis (Fig. 1).

When women were divided into those with histologic chorioamnionitis and those without histologic chorioamnionitis irrespective of gestational age or birth weight, women with histologic chorioamnionitis had higher proportion of babies with less than 10th percentile birth weight, crown heel length and head circumferences. The mean birth weight, length and head circumference were also lower in those with histologic chorioamnionitis (Table 2). In addition, women with histologic chorioamnionitis had significantly higher concentration of IL8 while the TNF α concentrations were comparable in both the groups (Table 2).

Twenty five of 180 (13.8%) women had bacterial vaginosis and the prevalence was not different between term normal, IUGR or PTD. Bacterial vaginosis was not associated with secretion of IL8 or TNF α from chorioamnion membranes.

TNF α was determined in 32, 56 and 58 term normal, IUGR and PTD respectively and was detectable in 60, 51 and 59%, respectively. The concentration of TNF α ranged from 2.70 to 234.80, 4.0 to 891.60 and 1.72 to 375.30 pg g^{-1} protein in term normal, IUGR and PTD respectively.

IL8 was determined in 31, 56 and 58 term normal, IUGR and PTD respectively and was detectable in all except 3 term normals. It ranged from 0.12 to 14.1 ng g^{-1} protein in term AGA, 0.07 to 23.9 ng g^{-1} protein in IUGR and 0.02 to 8.6 ng g^{-1} protein in PTD.

IL8 concentrations were increased in term normal and IUGR but more marked increase was observed in IUGR. When women were classified into high secretors (IL8 ≥ median) and low secretor (IL8< median) irrespective of gestational age and birth weight, mean birth weight of babies was 120 g lesser and mean crown heel length and head circumferences were lower in women with high secretion of IL8 compared to those who secreted below median (Table 3). In addition, women with symmetrical growth restricted babies had significantly higher secretion of IL8 in CA membrane.

Table 3: IL8 status in relation to new born anthropometry

IL8	BW (kg)	Length (cm)	HC (cm)	ABC (cm)	PI
<Median (71)	2.46±0.09	45.8±0.35	32.0±0.24	26.0±0.29	2.3±0.03
>Median (72)	2.34±0.07	44.8±0.40	31.7±0.29	25.8±0.30	2.4±0.03

Numbers in parentheses indicates number of subjects

DISCUSSION

The mean birth weight of babies in the present study was lower than those reported by Krishnaveni *et al*. (2005) and Mohan *et al*. (1990) while it was 100 g heavier than that reported by Indra *et al*. (1981). Similarly the crown heel length and head circumference were lower than that reported by Krishnaveni *et al*. (2005) but higher than that reported by Indra *et al*. (1981). As expected all the newborn anthropometric indices were significantly lower in term IUGR and PTD. Abdominal circumference (suggesting small viscera), fat percent and ponderal index were significantly lower in term IUGR babies, suggesting intrauterine growth restriction in most SGA babies. Moreover when we classified the newborns irrespective of gestational age, we found 15.8% to be symmetrically growth retarded, suggesting decreased growth potential beginning early in gestation in a high proportion of new borns.

The mean height and weight of the mothers in the present study were lower compared to studies reported from elsewhere in India, showing that the mothers delivering small babies are typically stunted, which could be due to poor nutritional status (Villar and Belizan, 1982).

Patients with histologic chorioamnionitis often have no clinical signs of infection, nevertheless, there are studies associating histologic chorioamnionitis with recovery of microorganisms such as Ureaplasma urealyticum, facultative and anaerobic bacteria from placenta (Hillier *et al*., 1988; Hecht *et al*., 2008). Romero *et al*. (2003) also showed recovery of bacteria from 72% of placentas with histologic chorioamnionitis. IL8 has also been shown to be correlated with isolation of bacteria from chorioamnion membranes (Jacobsson *et al*., 2005). Thus histologic chorioamnionitis and IL8 have been suggested as markers of infection of the chorioamnion membranes.

In the present study, we show the relationship of histologic chorioamnionitis with cytokine secretion from chorioamnion membranes and their association with IUGR in women delivered term. The results demonstrate association of high proportion of women with histologic chorioamnionitis with high concentration of IL8 and TNF α in chorioamnion membranes, irrespective of gestational age, unaccompanied by clinical signs of infection. The fact that IL8 was significantly associated with histologic chorioamnionitis, suggests subclinical infection as a significant cause for histologic chorioamnionitis in the present study. Moreover, the concentration of IL8 was considerably higher than that reported by Keelan *et al*. (1999) in women with normal delivery suggesting again a possible role for subclinical infection in stimulating higher concentration of IL8 in the chorioamnion membranes of subjects in the present study. All women in the present study belong to low socioeconomic status, which might explain higher proportion of histologic chorioamnionitis in women at term (Naeye, 1991). Intrauterine infection is well known to be associated with early preterm, but the preterms in the present study were all late (>34 weeks gestation) preterms, therefore link with histologic chorioamnionitis could not be established as shown elsewhere (Williams *et al*., 2000). There are many studies associating maternal infections during pregnancy with preterm delivery (Gibbs, 2001; Goldenberg and Rouse, 1998; Saini *et al*., 2003), however, very few studies have associated chronic intrauterine inflammation or infection such as malaria, with IUGR in term infants (Williams *et al*., 2000; Moormann *et al*., 1999). In this study babies born to mothers with histologic chorioamnionitis weighed 140 g lesser and were 1.2 cm shorter. Further more, it was interesting to note that higher proportion of mothers who delivered babies with Symmetrical Growth Restriction (SGR) had histologic chorioamnionitis, suggesting the possibility of histologic chorioamnionitis right from early pregnancy.

The observed effects of infection on birth weight have been suggested to be due to diminished uterine blood flow or even direct spread of infection to the placenta or amniotic fluid which may interfere with intrauterine growth or precipitate premature delivery. In addition to the direct effect of infections on uterine blood flow, infections are also associated with proinflammatory cytokine response that may affect intrauterine growth adversely and also promote PTD (Jacobsson *et al*., 2005; Shobokshi and Shaarawy, 2002).

BV which contributes to ascending genital tract infections and which has been shown to be an independent risk factor for infection of chorioamnion membranes (Jacobsson *et al*., 2005; Cauci *et al*., 2003) and early (<30 weeks) PTD was not associated either with histologic chorioamnionitis or PTD, which could possibly be due to absence of early PTD in the present study and also suggest a possibility of systemic infection contributing to histologic chorioamnionitis in the present study.

Around 90% of all LBW infants are born in developing countries and are caused by IUGR rather than PTD (Usha, 2004). Of the many factors that may affect birth weight, nutrition is the most widely studied and it is well known to have a profound effect on birth outcome (Singh *et al*., 1974). However infection during pregnancy, yet another important environmental factor, which could influence intrauterine growth has not been studied so far, due to lack of proper tools to diagnose infection in gestational tissues. The present study shows that intrauterine inflammation; a strong indicator of intrauterine infection is associated with fetal growth, while bacterial vaginosis is not associated. However, the association is not robust, which could be due to bias towards selecting a population that belongs to low socio economic status and thus high prevalence of chronic low grade infection resulting in higher prevalence of inflammation in all the women in the study. More controlled studies are required to delineate the role of infection/inflammation on intrauterine growth.

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