ORIGINAL RESEARCH

Case-control Association Study of TLR4 (rs 1927914) Polymorphism with the Risk of Low Birth Weight and Fetal Growth Restriction in North Indian Women

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Abstract

Background: Compared to newborns of normal birth weight at term gestation, the mortality and morbidity rates for low birth weight (LBW) and fetal growth restriction (FGR) babies are absurdly high. This is because these babies are more vulnerable to infections.

Aims and objectives: To study the association of toll-like receptor (TLR) 4 gene T>C (rs 1927914) polymorphism with the risk of LBW and FGR at term gestation in north Indian women.

Materials and methods: One hundred and eighty-two pregnant women (50 LBW and 32 FGR cases and 100 controls), 18–45 years of age, who attended the antenatal clinic or labor room were studied. We studied different maternal factors like maternal height, body mass index, number of antenatal visits, pre-pregnancy weight, and weight gain during pregnancy. In newborns, parameters like birth weight, gender, Apgar score after 1 and 5 minutes, NICU admission, and different anthropometric data were assessed. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was studied to analyze the single-nucleotide polymorphism of TLR4 (rs1927914) T>C.

Results: There was no significant association between TLR4 (rs 1927914) T>C polymorphism and risk of LBW and FGR. Genotype, TC, and CC of TLR4 T>C polymorphism showed a slight increase in the risk of LBW ($p = 0.38$).

Conclusions: The present study suggests that several inter-related factors increase the risk of LBW and FGR. The complex interplay and co-existence of many maternal and fetal factors are the leading cause of the increased risk of LBW and intrauterine growth restriction. Early prediction, identification of these risk factors, and proper management may prevent infant morbidities.

Keywords: Fetal growth restriction, Low birth weight, Single-nucleotide polymorphism, Toll-like receptors.

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Introduction

Innate immune responses to various microbial ligands and reactive host products depend on toll-like receptors (TLRs). A variety of pathogen-derived chemicals trigger innate immune responses when recognized by the membrane-bound TLRs.[1] All TLRs are expressed in a spatio-temporal manner at the maternal-fetal interface.[2] The outer (apical) plasma membrane, adjacent to the syncytiotrophoblast layer, expresses a high level of polarized TLR2 protein expression at 6–7 weeks of gestation. TLR3, TLR4, and their abundance in the cytotrophoblast layer form an effective TLR barrier.[3] Hofbauer and endothelial cells also express TLRs as an inherent defense mechanism during pregnancy.

Hofbauer cells have high levels of TLR2 and TLR4 expression, which allows them to respond to Gram-positive and Gram-negative bacteria, respectively, when exposed to these pathogens.[4] The chorioamniotic membranes express all 10 toll-like receptors.[5,6]

TLR4 is the most complex recruit and signals via both MyD88 and TRIF. Both TRIF and MyD88 pathways bring about IKK and lead to NF-kB activation via TNF-associated factor (TRAF) 6 activity. Subsequently, TRIF signaling gives rise to type I IFN production. The activation of MAPK and JNK by MyD88 signaling is observed.[7] Proinflammatory cytokines are produced at the maternal-fetal interface when TLR4 expression increases in immune or maternally derived cells. The growing fetus is put at risk by abnormal TLR4 activation.

The term LBW [birth weight (BW) <2500 gm irrespective of gestational age, sex, race, and clinical features] is entirely different from the FGR and small for gestational age (SGA).[8] Newborns with LBW have a greater risk of death in the immediate postnatal period than those with normal birth weight (NBW). Forty percent of the worldwide LBW burden comes from India; three-quarters of these babies are delivered at term.[9] The previous series highlighted the difference between LBW and NBW in terms of gene expression and proteins, including TLRs lined with innate immune function.[10]

An abnormally low fetal growth rate relative to the population or the individual's genetic growth potential is intrauterine growth restriction (IUGR) or fetal growth restriction (FGR). The terms FGR

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and SGA are often used synonymously, although there is a subtle difference between the two terms.[11] An estimated fetal weight (EFW) or abdominal circumference (AC) <10th percentile or <two standard deviations below the mean on the growth charts for the specific population and the specific gestational age is defined as SGA.

In contrast to most SGA babies in the FGR population, 50–70% of FGR fetuses are gestational age (AGA) but constitutionally undersized. Pregnant women with aberrant Doppler indices, such as an abnormally high umbilical or mean uterine artery pulsatility index (PI), are also considered FGR. Fetal growth restriction is a clinical definition irrespective of their BW percentile. Hence, AGA infants can be FGR if *in-utero* features of growth restriction and malnutrition are present at the time of delivery. Therefore, in the present case-control association study, we tried to evaluate the association between TLR4(rs 1927914) polymorphism with the risk of LBW and FGR in North Indian women.

Materials and Methods

A case-control association study was carried out from September 2017 to July 2019 in the Department of Obstetrics and Gynecology and the Department of Molecular and Human Genetics, Banaras Hindu University. The study group consisted of 182 antenatal females (50 LBW, 32 FGR cases, and 100 controls), 18–45 years of age, at term gestation, attending an antenatal clinic or labor room. Written informed consent was obtained from all the patients under study. University's ethics committee approval was also received before starting the study.

Inclusion Criteria

The cases comprised of pregnant females within the age group of 18–45 years attending an antenatal clinic or labor room of the Department of Obstetrics and Gynecology, SSH, BHU, delivering a baby that fulfilled the definition of LBW and FGR at term gestation. Pregnant women (18–45 years) delivering a baby with birth weight >2.5 kg at term gestation were included as controls.

Exclusion Criteria

Pregnant women at term gestation with the following complications were excluded from the study: multiple pregnancies, abnormal fetal karyotype, endocrine abnormalities, endometriosis, history of autoimmune disease, major fetal malformation, maternal infections like TORCH infection, and maternal diseases such as diabetes mellitus, previous or present high blood pressure, and kidney disease.

DNA Isolation

Genomic DNA from peripheral blood samples was isolated using the standard salting-out method. The concentration of DNA was measured by the spectrophotometric method (Nanodrop) by taking absorbance at 260 nm. The quality of DNA was evaluated by using 0.8% agarose gel.

Genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to assess the TLR4 (rs 1927914) T>C polymorphism. Polymerase chain reaction amplified the polymorphism region: 5 minutes at 940 °C, 35 cycles (30 seconds at 940 °C, 37 seconds at 60 °C, and 33 seconds at 720 °C), followed by a final extension for 10 min at 720 °C. Following primers were used, Forward: 5′-ACAAAATGGTCCCTCACAGC-3′ and Reverse: 5′-4 TGGAAAGTAGCAAGTGCAATG-3′. PCR product of 157 bp was digested with Sph1 restriction enzyme at 37 °C in a water bath for 18 hours. The digested product was separated on a 4% agarose gel. The T allele remains uncut by the enzyme, whereas the C allele gets cut and yields 90 bp and 67 bp products.

All the data were analyzed using SPSS (version 23.0; SPSS Inc., Chicago, IL, 11 USA). Data were summarized as mean and standard deviation for quantitative variables and frequency and percentages for categorical variables. The Chi-square test and Fischer exact test were used for categorical variables. Quantitative variables were analyzed using student's *t*-test and Mann–Whitney *U* test. Hardy–Weinberg equilibrium was observed for the SNP examined. According to the 95% confidence intervals, Odds ratio (ORs) were calculated and presented. Statistical significance was defined as a *p* value of 0.05 or lower.

Results

Compared to the control group, a significant association was observed between gestational age, pre-pregnancy weight, weight gain, and symphysio-fundal height with LBW and FGR. There was no significant association between maternal age with LBW and the FGR of the newborn compared to the control group (Table 1).

Low birth weight is more common in patients having short stature (<145 cm) (56%), body mass index (BMI) >25 kg/m^2 (76%), with antenatal visits <3 (68%) and 36% of patients having the previous history of LBW, as compared to control 9.0%. Moreover, FGR is more common in patients having BMI >25 (62.5%), antenatal visits <3 (53%), and 46.9% of patients having a previous history of FGR as compared to control 9%; however, no significant association is found between iron and folic acid tablets intake and effect of maternal height (Table 2).

Females with LBW babies mainly were illiterate (44%) and belonged to lower-middle socioeconomic status (68%). Fetal growth restriction was more common in illiterate females (37.5%) than in the control group (6%). In total, 65.6% of patients with FGR babies belong to the lower middle socioeconomic group than the

Table 1: Comparison of mean of maternal data in the three different group

Parameter	*Low birth weight (n = 50)*	*Fetal growth restriction (n = 32)*	*Control (n = 100)*	*p value*
Maternal age; years	25.00 ± 4.747	24.78 ± 4.172	24.80 ± 3.101	0.949
Gestational age; weeks	37.96 ± 1.212	37.94 ± 1.105	38.59 ± 1.326	0.004
Pre-pregnancy weight; kg	52.96 ± 7.321	55.44 ± 10.289	57.52 ± 4.994	0.001
Weight gain; kg	7.10 ± 1.972	6.09 ± 1.614	8.77 ± 1.090	<0.001
Symphysio-fundal height; cm	33.074 ± 2.1107	31.691 ± 2.0869	37.411 ± 0.8648	<0.001

Data expressed as mean ± SD

control group (38%). No association between the residence of the patient and the risk of LBW and FGR was observed (Table 3).

On comparing birth weight, Apgar after 1 min and after 5 min, mean HC, AC, CHL, and MAC in LBW baby group and control group, there were statistically significant (p <0.001) low mean values in LBW and FGR group as compared to controls respectively (Table 4).

The present study shows that genotype, TC, and CC of TLR4 T>C polymorphism show an increase in the risk of LBW. However, the values could not reach the significance level (Table 5).

There is no significant association of TLR 4 (rs 1927914) T>C polymorphism with the risk of FGR (Table 6).

Discussion

In the present study, numbers of patients belonging to the age group 18–20 years of age are high, with a risk of LBW (22%) and FGR (15.6%), as compared to those delivering NBW babies (9%). This highlights the risk of LBW and FGR with early maternal age. Madhur Borah et al. studied that teenage mothers have more chance of

Table 2: Distribution of different maternal factors in the comparison group

		Low birth weight		*Fetal growth restriction*		*Control*		
Study parameter		*No.*	*%*	*No.*	*%*	*No.*	*%*	*p value*
Maternal height	>145 cm	22	44.0	19	59.4	61	61.0	0.130
	<145 cm	28	56.0	13	40.6	39	39.0	
Body mass index	>25	38	76.0	20	62.5	89	89.0	<0.001
	<25	12	24.0	12	37.5	11	11.0	
ANC visit	≥3	16	32.0	15	46.9	58	58.0	0.011
	No or <3	34	68.0	17	53.1	42	42.0	
Iron and folic acid intake	Yes	34	68.0	22	68.8	74	74.0	0.696
	No	16	32.0	10	31.3	26	26.0	
Previous similar history	Absent	32	64.0	17	53.1	91	91.0	<0.001
	Present	18	36.0	15	46.9	9	9.0	
Previous abortion	No	36	72.0	23	71.9	90	90.0	0.026
	One	9	18.0	7	21.9	5	5.0	
	Two	3	6.0	1	3.1	5	5.0	
	Three	2	4.0	1	3.1	0	0.0	

Table 3: Distribution of different sociodemographic factors in the comparison group

		Low birth weight		*Fetal growth restriction*		*Control*		*p value*
Study parameter		*No.*	*%*	*No.*	*%*	*No.*	*%*	
Residence	Urban	33	66.0	24	75.0	69	69.0	0.688
	Rural	17	34.0	8	25.0	31	31.0	
Education	More than 10th	12	24.0	9	28.12	48	48.0	<0.001
	Up to 10th standard	16	32.0	11	34.38	46	46.0	
	Illiterate	22	44.0	12	37.5	6	6.0	
Socioeconomic status	Upper middle	16	32.0	11	34.4	62	62.0	<0.001
	Lower middle	34	68.0	21	65.6	38	38.0	

Table 4: Comparison of different new born data in the different groups

	Low birth weight (n = 50)	*Fetal growth restriction (n = 32)*	*Control (n = 100)*	*p value*
Birth weight; gm	2249.50 ± 179.151	2111.72 ± 246.487	2880.80 ± 160.892	<0.001
Apgar after 1 min	6.92 ± 1.104	6.66 ± 1.405	7.82 ± 0.411	<0.001
Apgar after 5 min	8.34 ± 0.939	8.19 ± 1.203	8.92 ± 0.273	<0.001
HC; cm	32.500 ± 0.9167	31.806 ± 0.9490	34.454 ± 0.5188	<0.001
AC; cm	30.386 ± 0.7931	29.856 ± 1.0555	33.336 ± 0.5016	<0.001
CHL; cm	45.664 ± 0.8504	45.119 ± 1.2408	49.672 ± 0.6453	<0.001
MAC; cm	9.260 ± 0.3213	8.909 ± 0.4409	10.416 ± 0.4790	<0.001

Data expressed as mean ± SD; AC, abdominal circumference; CHL, crown heel length; HC, head circumference; MAC, mid-arm circumference

Table 5: Distribution of genotypes and allele frequencies of TLR 4 (rs 1927914) T>C polymorphism in the study population

	Cases	*Controls*	*OR*	*CI*	*p value*
Polymorphism (genotype)					
TT	25 (0.50)	58 (0.58)			
TC	24 (0.48)	42 (0.42)	1.3	0.66–2.63	0.52
CC	1 (0.02)	0 (0)	6.8	0.27–174.13	0.6
Allele frequency					
T	74 (0.74)	158 (0.79)			
C	26 (0.26)	42 (0.21)	1.32	0.75–2.31	0.4

OR, odds ratio; CI, 95% confidence interval; Case, low birth weight (n = 50); Controls, healthy individual (n = 100); p value, Yate's corrected p value

Table 6: Distribution of genotypes and allele frequencies of TLR 4 (rs 1927914) T>C polymorphism in the studied population

	Cases	*Controls*	*OR*	*CI*	*p value*
Polymorphism (genotype)					
TT	22 (0.69)	58 (0.58)			
TC	10 (0.31)	42 (0.42)	0.6	0.26–1.46	0.38
CC	0 (0)	0 (0)			
Allele frequency					
T	54 (0.84)	158 (0.79)			
C	10 (0.16)	42 (0.21)	0.69	0.32–1.48	0.44

OR, odds ratio; CI, 95% confidence interval; Case, fetal growth restriction (n = 32); Controls, healthy individual (n = 100); p value, Yate's corrected p value

LBW.[12] Taywade et al. also reported that maternal age influences the incidence of LBW; moreover, the risk of LBW decreases with increasing mother age after 18 years.[13] The findings of Jawarkar et al. also agree with the present study, where maternal age showed a significant association with birth weight. While considering the age group 20–30 years as a reference category, odds of having an LBW baby are high in the age group below 20, indicating teenage pregnancy is a significant risk factor for LBW.[14] Ravikumar and Rajeshkannan from Tamil Nadu, conducted a study on 137 pregnant females found a significant association between maternal age and the risk of FGR.[15]

In our study, the LBW group had 38 (76%) patients having <8 kg of weight gain, 29 (90.6%) in the FGR group, and 49 (49%) patients having weight gain between 8.1 and 10 kg, followed by 45 (45%) belong to <8 kg weight gain. Hence, there was an association between lower weight gain during pregnancy with the risk of LBW and FGR (p value <0.001). Madhur and Borah et al. concluded that the risk of LBW is high in mothers with less weight gain during pregnancy.[12] According to Ravikumar and Rajeshkannan 2016, pre-pregnancy weight <45 kg was a significant risk but not an independent risk factor for FGR. Also, 64.2% of women have weight gain <10 kg during pregnancy, and 59.4% gave birth to FGR babies. This showed a significant association with the risk of FGR.[15]

The present series found no significant association between LBW and maternal height. Amosu et al. studied that females with having height <150 cm delivered LBW babies (2.33 ± 0.17 kg) compared to mothers with 150–154 cm height who delivered babies with more mean birth weight (2.47 ± 0.19), while taller mothers had heavier babies.[16]

Our study shows a significant association between body mass index number of ANC visits, history of previous abortion, and history of the previous low birth baby in the LBW group and FGR group, compared with the control groups, respectively. Fosu et al. studied the possibility of LBW in women not attending antenatal care as higher than in those who receive antenatal care even once (29.0% versus 20.4%).[17] Bugssa et al. concluded that the number of abortions and birth weight were not found to be statistically significant (p = 0.67).[18] Amosu et al. also found a statistically significant association in mothers with a previous history of stillbirths, abortions, and other poor pregnancy outcomes like LBW infants, perinatal mortality, and FGR when compared with females not having such history.[16] Ravikumar and Rajeshkannan studied that 50 out of the 135 multiparous women, 35 (25.9%) females had previously delivered FGR babies. Out of these 35, 21 (60%) gave birth to an FGR baby in the following delivery (which was in the study period itself). This was significant as a risk factor but not an independent risk factor.[15]

In the present study, we found that only 28% of mothers with babies in the LBW category, 34.4% in FGR one had hemoglobin (Hb) >11 gm/dL, but when compared to the control group, 53% had Hb >11 gm/dL. The study also shows the statistically significant association of anemia with LBW and FGR babies (p value = 0.044). Madhur Borah et al. from Assam concluded that the incidence of LBW was maximum (42.8%) in mothers not consuming iron and folic acid tablets during pregnancy. A significant association was noticed between birth weight and Hb of females during the antenatal period.[12] Ravikumar and Rajeshkannan found that anemia was very high-79% among the control group and 84.6% among mothers of FGR babies. Among anemic mothers born with FGR, 49.1% had mild, 50% had moderate, and 1 woman had severe anemia. Due to the high prevalence in both groups, anemia could not be statistically proven to be a risk factor.[15]

In the present series, a significant association was obtained between education and socioeconomic status with the risk of LBW and FGR. However, there was no association of the patient's residence with the risk of LBW and FGR. Taywade et al. reported a

low to medium standard of living index among cases (61.2%) than control (51.8%).[13] A meta-analysis assessing the risk factor of LBW reported socioeconomic status as a significant risk factor.[13] Borah et al. reported a higher risk of LBW babies among illiterate mothers (22.2%), and a significant association was noted between mothers' education and birthweight.[12] Kader and Perera also reported a higher risk of LBW babies among illiterate mothers.[19]

The present series showed a significant difference between the CHL, HC, AC, and MAC of different cases and the control groups (p value <0.05). Sakowicz et al. reported a significant association between CHL of both FGR and control group CHL.[20]

The present study does not show any significant association of TLR 4 (rs1927914) T>C polymorphism with the LBW and FGR group risk. Genotype, TC, and CC of TLR4 T>C polymorphism show a slight increase in the risk of LBW. There is no study, at present, showing the role of TLR in the prediction of LBW and FGR babies.

Conclusion

The present study does not show any significant association of TLR4 (rs 1927914) T>C polymorphism with the risk of FGR and LBW. Genotype, TC, and CC of TLR4 T>C polymorphism show a slight increase in the risk of LBW. However, the values could not reach the level of significance.

The present study suggests that several inter-related factors increase the risk of LBW and FGR. A complex interplay and co-existence of many maternal, fetal, and placental factors are the leading cause of the increased risk of LBW and FGR. Early prediction, identification of these risk factors, and proper management may prevent infant morbidities.

Our study had several limitations. First and foremost, LBW is likely the result of various underlying factors. To begin with, studies that look at many gene polymorphisms, rather than just a few selected ones, provide a more comprehensive picture of the etiology and biology of LBW in the family. Furthermore, we could recruit only 32 females who gave birth to FGR newborns because most of the pregnant females with the risk of FGR attending our antenatal clinic or labor room have a history of hypertension, diabetes mellitus, or any other systemic illness (belonging to exclusion criteria), and 5 females with the risk were lost to follow-up. We recommend conducting additional large-scale research to examine the genetic and environmental influences on immunity and inflammation to predict the likelihood of LBW and FGR.

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