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Research Article

The Genetic Analysis of Zinc Transporter (*Znt*) Gene Polymorphism and Its Association with the Risk of Preterm Birth

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ABSTRACT
Preterm birth (PTB) is the growing obstetric healthcare concern and is a dominant contributor to prenatal mortality and prolongs disability in the children of the Indian territories. Genetic variation in the functional genes is one of the causative factors for PTB. In present study, we investigated the association of the polymorphism in zinc transporter (*ZnT*) (*SLC30A1* and *SLC30A8*) with preterm birth (PTB = 72) and term birth (control, N = 138) in the Women of West India provinces. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was employed for genotyping of *SLC30A1* (C<G) and *SLC30A8* (A<G). The genotype distributions of *SLC30A1* (*P*< 0.001) showed significant difference between preterm birth cases and control women associated with higher risk of preterm birth. However, there was no relationship of *SLC30A8* (*G/G*) genotype with preterm birth indicated by insignificant (*P<0.68*) difference in genotypes between cases and control women. *SLC30A1 (G/G)* genotype was associated (OR= 0.07, 95% CI: 0.03-0.15, P<0.001) with higher risk of preterm birth in subjects with lower serum zinc content. Serum zinc was significantly declined at all three trimesters during pregnancy in women with preterm birth compared to control. The present findings indicated that the *SLC30A1* polymorphism would likely represent a genetic susceptibility factor and constitute a link for preterm birth.

KEYWORDS: Zinc Transporter, preterm birth, polymorphism, PCR-RFLP

INTRODUCTION

Zinc is an obligatory element due its structural, catalytic and regulatory function in human. Zinc insufficiency is most prevelent micronutrient deficiency observed in about 82% of all pregnant women around the word. Zinc scarcity during pregnancy could affect fetal growth and has a devastating effect on the offspring [1]. During fetus development process, a zinc insult is an essential factor in developing embryo, fetus or

neonate. Transfer of ample amount of zinc to the fetus is depending on maternal serum zinc level [2]. Maternal zinc deficiency may leads to a variety of maternal complications including preeclampsia, preterm birth, reduced birth weight, fetal neural tube defects and congenital malformations. Several epidemiological studies have confirmed the association of maternal zinc status with birth weight of the infant [3, 4]. Many nutritional, genetic and environmental factors may develop zinc insufficiency. Gastrointestinal disorder, phytate content of food could also affect the bioavailability of zinc and produced zinc scarcity. Decline utilization of zinc rather than intake of zinc poor diet are mostly responsible for maternal zinc deficiency [5]. The negative association has reported between maternal plasma zinc during pregnancy and birth weight of the infant in zinc-deficient women. Cervical maturation as well as maintenance of fetal and amniotic membrane required adequate amount of zinc during pregnancy [6]. It was reported that about 3-10% of protein is physiological active with zinc due to the presence of zinc binding domain. Passive transportation of zinc cannot be possible across the cell membrane, but Zn transporters mediate zinc uptake. Solute-linked carriers 30A (*SLC30A*, also called *ZnTs*) and *SLC39A (ZIPs)* are two major families of Zn transporter present in mammals. *SLC39A* zinc transporter is responsible for zinc uptake by the cell. *SLC30A* family is involved in the transport of zinc from the cytoplasm to extracellular space [7]. *SLC30A1* (*ZnT1*) and *SLC30A8 (ZnT8)* are cation diffusion transporter actively delivered maternal zinc into the embryonic environment [8]. Genetic variation in maternal genome accounts some disparity in preterm birth has been revealed in various candidate-gene studies [9]. Gene polymorphism in the toll-like receptor, matrix metalloproteinases (MMP) and tumor necrosis factor-α and Interleukins has been linked to spontaneous preterm birth [10].

However, It is documented that genetic defect in *SLC39A14 (ZIP14)* is not associated with Neural tube defects (NTDs) in a Turkish population [11] and with orofacial cleft-affected pregnancies in polish community [12]. Zinc deficient Turkish women had demonstrated a high prevalence of neural tube defect which was reversed by zinc supplementation [11]. Modification of epigenetic profile for progeny has observed in a zebra fish model due to parental zinc deficiency. Zinc deficient embryo shows altered expression of the gene including zinc transporter [13]. Genetic variations in *SLC30A* have an effect on the trasport of zinc from maternal serum into developing embryo which produces the risk of abnormal embryonic development and preterm delivery. The genetic component of zinc transporters is still currently unable to identify as a risk factor for preterm birth. So, we aimed to study the *SLC30A* gene defects and its possible role in the pathogenesis of preterm delivery

MATERIALS AND METHODS

Study subjects

The investigation was started after acquiring ethical approval by the Institutional Ethics Committee at Shri Alpesh. N. Patel Post Graduate Institute of Science and Research, Anand, Gujarat. The present case-control study consisted of 210 women, comprising 72 preterm and 138 controls who attended at the Department of Obstetrics and Gynaecology of Akanksha and Garima Hospital at Anand and Dr. Padma Gynaecology Hospital at Vadodara, Gujarat state, India from October 2015 to May 2017. Mothers with uncomplicated normal delivery between 38 and 41 weeks were considered as control group, whereas the preterm group consisted of mothers, who delivered their babies between 24 and 36 weeks. Gestational age was calculated from the date of last menstrual period and also validated by ultrasonography. Information about the patient's demographic, obstetric history, life style, family history, ethnicity of the participants has been noted. The birth weight of new infants was recorded within an hour of their birth. Moreover, Informed consents were received from the participants. Exclusion criteria of the study were an abnormal fetus, preeclampsia, maternal age >35 years, stillbirth, preeclampsia, hypertension, history of tuberculosis, diabetes and renal disease.

Isolation of genomic DNA and Biochemical measurement

Fresh collected venous blood was used for high quality extraction of genomic DNA by genomic DNA extraction kit (OmniPrep™ G-Bioscience Kit, Catalogue number: 786-136, 786-136S). The integrity of the genomic DNA was evaluated by

0.8% agarose gel electrophoresis. The purity of DNA was estimated by A260/A280 proportion in the experiment. The DNA samples were stored at -20°C for further analysis. Assay of serum Zn concentrations was carried out by Double beam spectrophotometer (systronics) using commercial kit (Tulip Diagnostics Pvt. Ltd, Goa. Cat. No. : 1103130150).

SNP Selection and Genotyping

The detection of single nucleotide polymorphism in *SLC30A1* and *SLC30A8* gene were carried out by PCR amplification (Veriti Thermal Cycler 96 Well) and restriction digestion of the products with *TaqI* and *PstI* (Thermo Fisher Scientific Inc.) in the preterm cases and controls. The primers used for specific amplification were listed in Table 1. The PCR reaction was carried out with 25 µl solutions consisted of 1X buffer, $MgCl_2$ 1.0 mM, 1.0 µl template DNA, and each 0.5 µl of forward and reverses primers, 12.5 µl PCR Master Mix and 10.5 µl ddH_2O for each gene. 643 bp segment of *SLC30A8* was amplified using PCR under the following conditions: 9 cycles- 96°C, 35s; 56°C, 45s; and 35s at 72°C followed by 25 cycles: 96°C, 25s; 52°C, 50s; 72°C for 40s and 10 additional cycles: 72°C, 5 min; 50°C, 1.0 min and 72°C at 1 min and 30s. PCR reaction conditions for *SLC30A1* started with an initial denaturation of 5 min at 94°C followed by first 5 cycles-45 second at 94°C, 60 second at 64°C, 2 minute at 72°C then 25 cycles- 30 second at 94°C, 30 second at 64°C, 45 second at 72°C;5 min extension at 72°C.

Table 1: Primer sequences for ZnT *SLC30A1* and *SLC30A8* polymorphisms amplification

Locus	Primer sequence	Length
SLC30A8	Forward Primer: 5' GAAGTCCAGGTTCCAAACCA 3'	643 bp
ZnT8	Reverse Primer: 5' AAGGAGACCAAGTCAGGAA 3'	
SLC30A1	Forward Primer: 5' AGTACAGGTAAAGGCCAAGGT 3'	352 bp
ZnT1	Reverse Primer: 5' TTGATTTGTGGTTTTAAGGTAGG 3'	

The amplified fragment of *SLC30A8* genotype was digested by 0.5µl restriction enzyme *Pts1* at 37°C for four hr. Amplified *TaqI* polymorphic segment of *SLC30A1* (352bp) were digested for overnight at 55°C with *TaqI* restriction enzyme. The gel was imagined under UV light using a Trasilluminator system (Genie, Bangalore, India). The results were further confirmed by 12 % polyacrylamide gel electrophoresis (PAGE) with silver staining.

STATISTICAL ANALYSIS

The data of genotype frequencies of the ZnT gene polymorphism in studied population were analysed using the computer software SPSS (version 20). The association of genetic polymorphism with preterm birth was performed using Fisher's exact test. A $P<0.05$ was considered statistically significant. Odds ratio (OR) with 95% confidence interval was calculated to determine the relative risk of preterm birth. $P<0.05$ is a standard to determine the significant difference.

RESULTS

The demographic characteristics of total 210 participants (72 in the preterm birth group and 138 in control group) are shown in Table 2. Subjects of preterm group exhibits significantly ($P < 0.05$, Table 2) lower level of serum zinc compared to control group with term birth. In this study, there was no difference in maternal ages ($P = 0.24$) and maternal BMI ($P = 0.42$) between women in control group and the preterm group. The mean birth weight of control and cases were 3362.4 ± 442.4 and 2265.8 ± 526.7 g respectively. There was a significant difference ($P < 0.05$) in initial birth weight of newborn baby delivered by control and preterm group.

The genotype frequencies distribution of *SLC30A1* and *SLC30A8* polymorphism in both groups were consistent with those predicted by Hardy-Weinberg equilibrium shown in Table 3. The genotypic pattern of *SLC30A8 (A>G) and SLC30A1* (C>G) polymorphism obtained from PCR-RFLP are presented in Fig. 1 and Fig. 2 respectively. As for GG gene type frequency of *SLC30A1* (C>G) polymorphism, the cases was higher than the control (83.33% cases vs. 43.2% control) and the difference has statistical significance. The genotype *SLC30A1 C>G* has an odd ratio 4.32 (95% Confidence Interval: 2.14 – 8.75) which indicate a high risk of preterm birth in the presence of allele G, both in codominant and recessive models. The genotype distribution

of the *SLC30A8 (A>G)* polymorphism did not differ significantly among preterm birth compared to control. The gene frequency of the *SLC30A8 (A>G)* polymorphism was not significantly associated with susceptibility to PTB in subjects. In addition, the significant difference in serum zinc has been noted among women with preterm birth compared to the control during the second trimester of pregnancy (Fig.3).

However, the maternal *SLC30A1* GG variant was associated ($P < 0.001$) with lowered serum levels of zinc than the CC variant, and perhaps with increased risk for the preterm delivery in pregnant women. We did not find significant effect of *SLC30A8 (A>G)* polymorphism on maternal zinc status and pregnancy outcomes.

Table 2: Demographic features of 138 term (control) and 72 preterm subjects

Characteristics	Control	Preterm
Maternal age	27.5 ± 3.5	29.2 ± 3.8
Maternal BMI (kg/m2)	24.9 ± 4.2	22.8 ± 4.6
Previous miscarriage (%)	06	10
Birth weight (g)	2265.8 ± 526.7	3362.4 ± 442.4*
Hemoglobin (g %)	10.36 ± 3.75	9.87 ± 4.23
Serum Zinc (µg/dl)	64.9 ± 17.5	48.6 ± 13.64*

* indicate a significant difference at the 5% level.

Table 3: The genotype and allele distribution of ZnT (*SLC30A1* and *SLC30A8*) polymorphism between preterm cases and control subjects

SNPs	Genotypes	Control N (%)	Preterm N (%)	Odd ration (95% CI)	P value
ZnT (SLC30A1)					
	CC	74 (53.62)	12 (16.67)		
	CG	47 (34.05)	18 (25.00)	0.42 (0.19-0.96)	0.041
	GG	17 (12.31)	42 (58.33)	0.07 (0.03-0.15)	0.001
	CG + GG	64 (43.0)	60 (83.33)	4.32 (2.14-8.75)	0.001
ZnT (SLC30A8)					
	AA	44 (31.88)	21 (29.17)		
	AG	52 (37.68)	28 (38.89)	0.89 (0.44-1.77)	0.73
	GG	42 (30.43)	23 (31.94)	0.87 (0.42-1.80)	0.71
	AG+GG	94(68.11)	51(70.83)	0.88 (0.47-1.64)	0.68

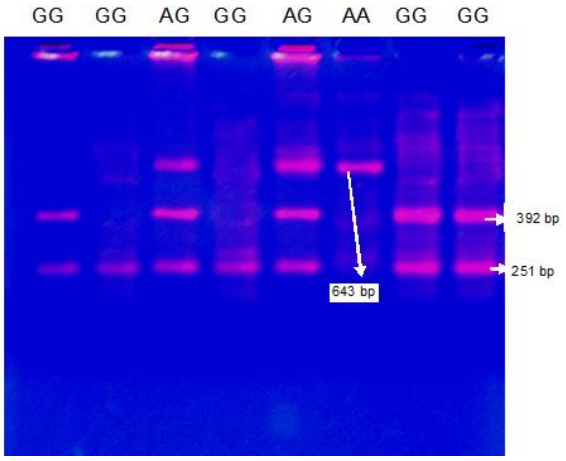


Fig 1: Characteristic of polyacrylamide gel for SNP *SLC30A8* (A/G) by PCR-RFLP SNP. Lines are numbered form left to right. Homozygous (A/A) (lane 2, 3), homozygous (G/G) (lane 1, 4, 6) and heterozygous (A/G) (Lane 5, 7, 8) are observed.

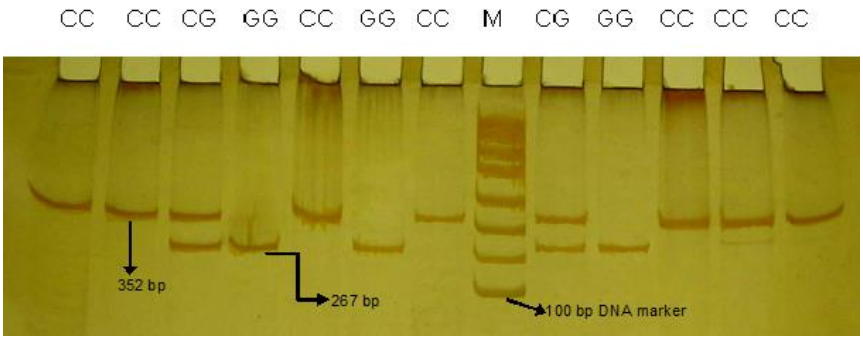
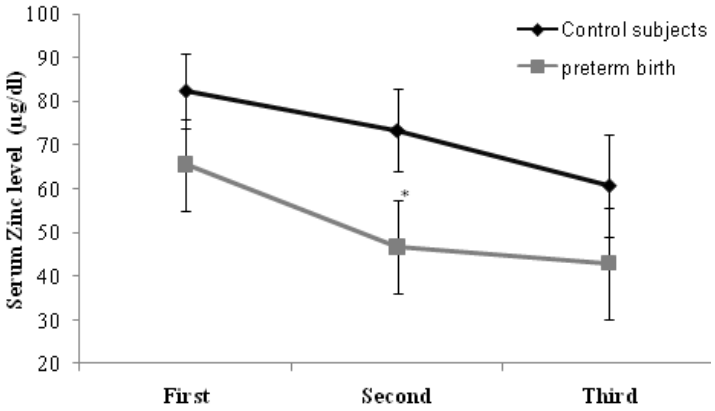


Fig. 2: Genotyping of zinc transporter gene *SLC30A1 gene* polymorphism observed using PCR-RFLP technique.



* indicate a significant difference at the 5% level.

Fig. 3: Zinc level in serum at each trimester during pregnancy of control women and subject with preterm birth.

DISCUSSION

Zinc deficiency during pregnancy in the mother has been related to adverse fetal outcome. Bioavailability of zinc in the diet is influenced by not only food and dietary supplement but also by a genetic mutation in zinc transporter. Zinc transporter such as *ZnT* that are localized on the plasma membrane of the placenta may play an important role in zinc uptake to developing fetus [14, 15]. The cause of premature delivery is multifunctional but genetic predispositions are essential contributor. Maternal genome plays a significant role in genetic contribution to preterm birth [10]. In the last decade, genetic polymorphism in *ZnT* transporter genes may dysregulate expression and activity of transporters implicated in preterm birth and other chronic diseases [16]. We report that the zinc insufficiency has a significant interaction on the association between the *SLC30A1* polymorphisms and the risk of a PTB. In zinc deficient women, the *SLC30A1 GG* genotype was associated with an increased risk of a PTB, whereas *SLC30A8 (A>G)* polymorphism was not associated with PTBs in present study. Our result shows that subjects with the *G* allele of *SLC30A1* were more likely to experience preterm birth than control subjects. According to several earlier reports, single nucleotide polymorphisms (SNPs) in candidate genes exhibit their association with the PTB [17]. *SLC30A1* is not the first genetic factor suspected in preterm birth in humans. However, it has been previously shown that ancestral allele of TNF-α, IL6, MTHFR, MMP are associated with the PTB [18]. Expression of zinc transporter in small intestine and liver maintain a sufficient amount of zinc supply to developing fetus and neonate [19]. *ZnT1* (*SLC30A1*) is ubiquitously expressed in the intestine and regulates the absorption of dietary zinc. *ZnT1* is also expressed in villone visceral splanchnopleure of the placenta [20]. *SLC30A1* has a critical function in regulating the transport of maternal

zinc to developing an embryo (Ford 2004). Also, embryonic lethality observed in *ZnT1* null mice indicated a significant role of *Znt1* in transporting zinc to embryo for healthy development [8]. Polymorphism in *ZnT1* leads to decreased expression of transporter and declining dietary zinc intake. The present study showed that maternal serum zinc concentration was significantly lower in preterm cases than that of control which might be due to polymorphism in *SLC30A1* of zinc transporter. Decreased dietary absorption of zinc also leads to zinc deprivation in maternal blood during pregnancy. Numerous of human and animal studies have been summarized poor maternal zinc status and pregnancy complication or preterm birth [21, 22]. In most instances, transient zinc deficiency in an infant has been reported due to the mutation in *SLC30A2* gene of maternal genome [23]. We also found that serum zinc level was consistency declined from first to the third period of gestation in all participated subject. Sikorski [24] has reported significantly lowered maternal zinc index in a patient with preterm delivery than in patients without this complication which is in agreement with the result of the present study.

CONCLUSION

In summary, it is biologically plausible that SNPs in *ZnT1* (*SLC30A1*) may have effect on individual susceptibility to preterm birth. The obtained information could be used for screening and treatment of women at risk of preterm birth complication to reduce the risk of specific congenital disabilities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this research article.

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