Original Article

Effect of fluoride on the uteroplacental transfer of calcium and magnesium and their role in congenital anomalies in newborns

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ABSTRACT

Introduction: Calcium and magnesium are one of the most important micronutrients for fetal development. Environmental pollutants like excess fluoride can hamper the action of calcium and magnesium resulting in a bad outcome of pregnancy.

Aim: We aimed to find out the effect of fluoride on the uteroplacental transfer of calcium and magnesium and the role of these minerals as a causative factor of congenital anomalies in newborns.

Materials and Methods: Out of 50, 25 newborns with congenital anomalies were included in group I and 25 healthy newborns were included in group II. Cord blood fluoride was estimated by the ion-selective electrode while calcium and magnesium were estimated by the autoanalyzer. Unpaired '*t*' test and Pearson's correlation test were applied for statistical analysis.

Results: Serum fluoride levels were significantly raised and serum calcium levels and serum magnesium levels were significantly decreased in newborns with congenital anomalies as compared to newborns without congenital anomalies ($P = 0.000$). Serum fluoride levels showed a positive correlation with serum calcium in group II, which got inverted in group I. Both the results were statistically significant. Serum fluoride levels showed a positive correlation with serum magnesium levels in group II, which got inverted in group I. Both the results were statistically insignificant.

Conclusion: Hypocalcemia and hypomagnesemia can affect fetal development. Environmental pollution due to fluorosis emerges as a factor as fluoride has a direct influence over calcium and magnesium absorption and transfer via the placenta to the developing fetus. Prophylactic measures have to be taken to counter the effect of fluorides on calcium and magnesium for proper development of the growing fetus.

Key words: Calcium; congenital anomalies; fluoride, magnesium; placental transfer.

Introduction

Adequate maternal nutrition is important for optimal fetal growth and development. Supplementation with multiple micronutrients has been demonstrated to improve fetal growth in undernourished populations.[1] Calcium is one important micronutrient responsible for different cellular functions, maintaining tissue strength and structure and, in particular, bone integrity.[2] Pregnancy is a state of high calcium requirement, inadequate maternal calcium intake may adversely affect fetal growth and bone mineralization.[3] Magnesium is primarily found within the cell where it acts as a cofactor for more than 300 enzymatic reactions. It critically stabilizes enzymes, including many ATP-generating reactions and contributes to the regulation of vascular tone, heart rhythm, platelet-activated thrombosis, and bone

Access this article online	
	Quick Response Code
Website: www.tjogonline.com	
DOI: 10.4103/TJOG.TJOG_42_19	

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How to cite this article: Ghosh A. Effect of fluoride on the uteroplacental transfer of calcium and magnesium and their role in congenital anomalies in newborns. Trop J Obstet Gynaecol 2020;37:114-8.

Received: 17-05-2019 **Accepted:** 25-03-2020
Published Online: 14-08-2020

formation.[4] Magnesium deficiency during pregnancy can induce maternal and fetal nutritional problems along with lifelong consequences. Magnesium deficiency is associated with uterine hyperexcitability,[5] premature labor, and preeclampsia. It may also result in sudden infant death syndrome (SIDS)[6] and intrauterine growth retardation (IUGR). Magnesium is an essential element for fetal well-being and supplementation of magnesium may be benefited to fetal outcome.

A trace amount of fluorine is required for tooth and bone development. A very small amount of fluoride in food and drinking water promotes normal bone development, increases retention of Ca^{++} and $PO_{4,}$ and prevents old age osteoporosis. Fluoride can inhibit the function of isolated osteoclasts. Fluorine stimulates osteoblast and causes an increase in calcium deposition and increased density of bone.[7]

Excess of fluoride exposure has its detrimental effects such as low birth weight, stillbirth, neonatal death, and congenital anomalies. In India, excess fluoride problems have been detected in 20 states and arsenic in two states. A study has been reported regarding serum fluoride levels in neural tube defects and they observed higher levels of fluoride levels in these infants.[8,9] Besides, few studies have observed significantly higher fluoride levels in osteosarcoma.[10,11] Studies conducted in Brazil and China have shown that children whose parents use pesticides in and around the home are more likely to get leukemia, brain cancer, non-Hodgkin's lymphoma, and soft tissue sarcoma. Environmental Health Prospective Journal study has shown that children exposed prenatally to household insecticides had an increased risk of brain cancer later on. A few studies of human populations have suggested that fluoride might be associated with alterations in reproductive hormones, fertility, and Down's syndrome.[12]

The association between maternal serum levels of various elements such as calcium, magnesium, phosphorous, and fluoride and birth outcomes is complex and is influenced by many biologic factors. Understanding the relationship between maternal serum levels of these elements and birth outcomes may provide a basis for developing nutritional interventions that will improve birth outcomes and long-term quality of life and reduce mortality, morbidity, and healthcare costs.

Therefore, the current study was planned to observe the relation between calcium, magnesium, and fluoride as a causative factor for birth anomalies in newborns.

Materials and Methods

The present study was conducted in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynecology, Pandit Bhagwat Dayal Sharma PGIMS, Rohtak. After giving complete information regarding the study in a verbal and written format both in English and Hindi, proper informed consent was taken from the study participants. Fifty babies delivered in the labor room were divided into two groups. Twenty-five newborns with visible congenital anomalies were included in group I. Only babies with visible congenital anomalies were taken as inclusion criteria for this group. This group was compared with group II, which were taken as controls and comprised of 25 healthy newborns without any congenital anomalies.

All the patients were subjected to a detailed history and clinical examination as per proforma attached. About 10 mL cord blood was collected from the placental end of the umbilical cord immediately after delivery of the baby. The serum was separated by centrifugation and routine investigations were done on the same day and serum for special investigations was preserved at −20°C till analysis.

- Serum fluoride levels were measured by the ion-selective electrode (ISE).[13]
- Serum calcium and magnesium were measured by the kinetic method using Randox Suzuka closed system kit.[14]

Results were expressed as mean ± SD and unpaired '*t*' test and Pearson's correlation test was applied. Data were considered to be significant if $P < 0.05$.

Results

Cleft lip ($n = 6$) was the most common congenital anomaly in the present study while CTEV was the second most common ($n = 4$). A limb defect (DDH) was seen in three cases. Hypospadias, polydactyly, and spina bifida showed a similar distribution with two cases each omphalocele, anencephaly, gastroschisis, encephalocele, cleft palate, and imperforate anus were seen in 1 case each.

In the present study, the birth weight of babies with congenital anomalies was lower than the babies without congenital anomalies and the difference is statistically significant. Gestational age was also lower in group I as compared to group II but it was not statistically significant.

The musculoskeletal system is the most commonly involved system followed by the orofacial system.

Serum fluoride levels were significantly raised in newborns with congenital anomalies (group I) as compared to newborns without congenital anomalies (group II). Serum calcium levels and serum magnesium levels were significantly decreased in newborns with congenital anomalies (group I) as compared to newborns without congenital anomalies (group II).

Serum fluoride levels showed a positive correlation with serum calcium levels in group II, which got inverted in group I. Both the results were statistically significant.

Serum fluoride levels showed a positive correlation with serum magnesium levels in group II, which got inverted in group I. Both the results were statistically insignificant.

Discussion

Developmental processes from fertilization to organ formation and function are dependent on the dynamic release of calcium ions. Human developmental disorders may occur due to defects in the regulation of calcium-sensitive factors. In Down's syndrome, disruption of Ca^{2+} regulation of the calcineurin/nuclear factor of activated T cell (NFAT) pathway results in Down's syndrome, which is further supported by the conservation of pathways regulating NFAT nuclear localization, namely activation by intracellular Ca^{2+} increase and calcineurin and inhibition by dual specific tyrosine phosphorylation regulated kinases (DYRK). Another congenital anomaly named Noonan syndrome, which is associated with facial dysmorphia, disproportionate short stature, increased risk of leukemia and congenital heart defects is thought to be caused by mutations in an src homology 2 containing protein tyrosine phosphatase non-phosphatase type 2 (SHP-2/PTPN11), which cause its constitutive activation.[15] On the other side, it has been shown that the use of calcium channel blockers and beta-blockers do not cause any risk of developing embryo.[16] In the present study, serum calcium levels were significantly decreased in newborns with congenital anomalies (group I) as compared to newborns without congenital anomalies (group II, Table 1). An important relationship exists between maternal serum calcium levels and neonatal calcium homeostasis. Normal fetal and neonatal calcium homeostasis dependent on adequate maternal calcium supplies. Maternal hypercalcemia can suppress fetal parathyroid hormone (PTH) function causing neonatal hypocalcemia and conversely, maternal hypocalcemia can stimulate fetal PTH causing bone demineralization[17]

In the present study, serum magnesium levels were significantly decreased in newborns with congenital anomalies (group I) as compared with newborns without congenital anomalies (group II, Table 1). Magnesium has an established role in obstetrics and deficiency of magnesium may be associated with preeclampsia and preterm delivery and possibly with low birth weight. Magnesium deficiency during pregnancy has been documented to increase neonatal morbidity and mortality.[18] The findings of our study also showed that the birth weight of the babies with congenital anomalies was significantly less than the healthy babies in the control group [Table 2]. There is an increased demand for magnesium during pregnancy. Evidence indicates that magnesium status is associated with pathological incidents in pregnancy and its deficiency increases the incidence of preterm birth and spontaneous abortion during pregnancy.[19] Kisters *et al*.[20] have characterized significant differences in placental gene expression for several magnesium transporter systems in pregnancy and preeclampsia women. Low postpartum magnesium status is a significant indicator of the development of type 2 diabetes in women with GDM.[21]

In the present study, cleft palate was the most common type of congenital anomalies encountered. But congenital defects related to the musculoskeletal system was the most commonly involved system [Table 3]. This can be due to the hypocalcemic and hypomagnesemia state of the mother during pregnancy, which has a direct role in the development of the musculoskeletal system.

Serum fluoride levels were significantly raised in newborns with congenital anomalies (group I) as compared to newborns without congenital anomalies (group II, Table 1). Serum fluoride levels showed a positive correlation with serum calcium levels in group II, which got inverted in group I [Table 4]. Both the results were statistically significant. Many studies reported the occurrence of hypocalcemia during fluorosis. It might be due to decreased calcium absorption from the gut. Due to high fluoride intake, insoluble calcium fluoride salt is formed in the intestine and gets excreted through feces leading to hypocalcemia mimicking a decreased intake of calcium. This hypocalcemia may lead to fetal parathyroid stimulation causing an imbalance of calcium and phosphate. This hypocalcemia may cause different types of skeletal abnormalities during developmental stages.[22] The findings of our study were also in agreement with the hypothesis that high fluoride contents can inhibit calcium transport.

Serum fluoride levels showed a positive correlation with serum magnesium levels in group II, which got inverted in group I [Table 5]. Both the results were statistically insignificant. The toxic effect of fluoride ion plays a key role in acute magnesium deficiency. In an animal study, it has been found that lowest magnesium-containing diets

Table 1: Serum (Cord blood) fluoride, calcium, magnesium levels in both groups (Mean±SD)

	Group I	Group II	*P*
Fluoride (mg/L)	0.19±0.051	0.071±0.035	0.000
Calcium (mg/dL)	7.03±1.05	8.78±1.09	0.000
Magnesium (mg/dL)	1.52±0.52	2.12±0.38	0.000

$P<0.05$ had been taken as significant

Table 2: Comparison of birth weight in both groups (Mean±SD)

	Group I	Group II	*P*
Weight (kg)	2.37±0.41	2.59±0.26	0.003
Gestational Age (Weeks)	36.10±1.99	36.27±1.68	0.314

$P<0.05$ had been taken as significant

Table 3: Distribution of cases according to the system involved

System involved	Numbers of cases
Musculoskeletal system	12
Orofacial system	7
Central nervous system	2
Genitourinary system	2
Gastrointestinal system	2

Table 4: Correlation of levels of serum fluoride levels and serum calcium levels in two groups (Mean±SD)

	Group I		Group II	
Fluoride (mg/L)	0.19±0.051	r=-0.436 P=0.002	0.071±0.035	r=0.306 P=0.03
Calcium (mg/dL)	7.03±1.05		8.78±1.09	

$P<0.05$ had been taken as significant

Table 5: Correlation of levels of serum fluoride levels and serum magnesium levels in two groups (Mean±SD)

	Group I		Group II	
Fluoride (mg/L)	0.19±0.051	r=−0.250 P=0.079	0.071±0.035	r=0.149 P=0.301
Magnesium (mg/dL)	1.52±0.52		2.12±0.38	

$P<0.05$ had been taken as significant

have significantly more fluoride deposition in bone and teeth, whereas diets containing 5 times normal magnesium have less fluoride deposition indicating a highly significant, level-dependent interaction between magnesium and fluoride. Fluoride retention values simply reflect absorptive changes, which indicates that the site of the interaction between magnesium and fluoride is at the intestinal level, most likely involving insoluble complex formation.[23]

It has been suggested in various studies that the placenta acts as a barrier to fluoride transport to the fetus. In 1955, Feltman and Kossel observed much higher concentrations of fluoride in peripheral regions (in comparison with central ones) of two examined placentas.[24] They suggested that this differentiation was closely related to the calcium content of these parts of the tissue. Another study suggested that fluoride accumulation in the placenta may be connected with local focuses of calcification.[25] It has also been hypothesized that higher concentrations of fluoride on the peripheral part of the placenta may be due to higher levels of calcium and magnesium in that region. Bruns *et al*.[26] have reported the presence of a calcium-binding protein in mouse placenta and studied its regulation and showed that the fetal levels of calcium were unchanged with higher ingestion of calcium but the placental calcium concentrations and calcium-binding protein levels were significantly increased. Thus, the higher calcium concentrations in the placenta may be attributed to the presence of calcium-binding protein which indirectly may be responsible for the higher fluoride concentrations in the placenta when compared with the maternal and cord sera. A similar magnesium transporter was also reported which transports magnesium across the placenta to the fetus.[27] In all animal species studied, calcium and magnesium were higher on the fetal compared to the maternal side of the placenta.[28] It has been seen that the concentrations of these ions are highest on the marginal part of the placenta. Hence, the placenta can act as a regulatory barrier for the transport of various micronutrients to the fetus from the maternal circulation.

Conclusion

Micronutrients are essential elements for the development of the fetus. In the present study, it has been found that hypocalcemia and hypomagnesemia are associated with the bad outcome of pregnancy in terms of congenital anomalies. The development of hypocalcemia and hypomagnesemia can be multifactorial. Environmental pollution due to fluorosis can also emerge as a factor as fluoride has a direct influence over calcium and magnesium absorption and transfer via the placenta. The detailed mechanism by which fluoride can cause hypocalcemia and hypomagnesemia is yet to be explored. Moreover, the requirement of prophylactic administration of calcium and magnesium during pregnancy has to be considered to prevent the occurrence of bad outcomes of pregnancy. The findings of the present study may serve as baseline data for planning preconception and nutrition-based interventions during pregnancy to improve pregnancy outcomes.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship
None.

Conflicts of interest
There are no conflicts of interest.

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