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ASSESSING THE IMPACT OF FLUORIDE IN DRINKING WATER ON CALCIUM AND VITAMIN D, IN PREGNANT WOMEN

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LIST OF ABBREVIATIONS

Abbreviation	Descriptions
PPM	Parts Per Million
VIT D	Vitamin D
Ca++	Calcium
Fl	Fluoride
BMD	Bone Mineral Density
WHO	World Health Organization

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ABSTRACT

Introduction: Fluorine, a highly reactive element naturally present in air, water, and food, is mainly ingested through drinking water. The American Academy of Pediatrics recommends a daily fluoride intake of 0.05–0.07 mg/kg body weight for dental health. Excessive fluoride exposure, however, may disrupt calcium balance and reduce vitamin D levels, which are essential for maternal health during pregnancy. High fluoride levels in drinking water can alter calcium transport and reduce vitamin D levels, potentially impacting maternal health by disrupting calcium homeostasis and vitamin D metabolism. The primary objectives were to assess fluoride concentrations in drinking water, serum, and urine samples, and to examine the relationship between fluoride exposure and serum calcium and vitamin D levels among pregnant women nearing delivery. **Methodology:** A cross-sectional, comparative study was designed, enrolling 180 pregnant women aged 18–45 with no prior history of hypercalcemia, renal conditions, or significant pregnancy complications. Participants were recruited from tertiary care hospitals in Karachi (high fluoride) and Faisalabad (low fluoride). Blood and urine samples were collected approximately one month before delivery to measure serum fluoride, calcium, and vitamin D levels. Water samples from both regions were collected and analyzed to determine fluoride concentrations, with values exceeding **Finding:** The results revealed that pregnant women in the high-fluoride group (Karachi) had significantly lower mean serum calcium (8.8 ± 0.5 mg/dL) and vitamin D (21.3 ± 4.2 ng/mL) levels compared to those in the low-fluoride group (Faisalabad), where mean calcium and vitamin D levels were 9.6 ± 0.4 mg/dL and 27.6 ± 3.8 ng/mL, respectively ($p < 0.001$). This inverse association between fluoride concentration and calcium/vitamin D levels persisted despite similar dietary and supplementation patterns between groups. Socio-demographic factors and lifestyle variables were also examined, **Conclusion:** The findings indicate that high fluoride exposure in drinking water is associated with decreased serum calcium and vitamin D levels among pregnant women. These deficiencies, if unaddressed, may compromise maternal health and fetal development. The study emphasizes the need for fluoride mitigation efforts in high-exposure areas and advocates for tailored nutritional interventions to address calcium and vitamin D deficiencies among pregnant women in fluoride-affected regions.

Keywords: Fluoride, Vitamin D, Calcium, Pregnant Women, Drinking Water.

INTRODUCTION:

Fluorine, the most electronegative element, readily forms fluoride ions, which are commonly present in drinking water. [1] While fluoride in optimal amounts supports dental and bone health, excessive exposure can cause adverse health effects, including dental and skeletal fluorosis, as well as impacts on kidney, nerve, and muscle function. According to the American Academy of Pediatrics, a safe fluoride intake for dental health benefits is 0.05 to 0.07 mg/kg of body weight per day. [2] However, elevated levels of fluoride in drinking water may disrupt essential minerals, including calcium and vitamin D, which are critical for maternal health, particularly during pregnancy. [3] Fluoride's potential impact on calcium regulation has been studied since 1948, as fluoride ions can interfere with calcium transport mechanisms and decrease calcium absorption, affecting bone mineralization and overall cellular function. Vitamin D is vital for maintaining calcium and phosphorus levels in the blood, supporting skeletal development, and enhancing maternal and fetal bone health. Deficiency in vitamin D during pregnancy has been linked to compromised bone growth, shorter gestational periods, and increased risk of hypertensive disorders such as preeclampsia. [4] Sufficient levels of vitamin D and calcium are essential to meet the heightened physiological demands of pregnancy, as maternal calcium levels are modulated to support fetal bone mineral accumulation, particularly in the third trimester when calcium transfer to the fetus is most significant. [5] Calcium homeostasis during pregnancy is regulated by parathyroid hormone (PTH), which plays an essential role in balancing calcium and facilitating placental calcium transfer. Low calcium levels in pregnant women have been linked to adverse pregnancy outcomes, underscoring the importance of maintaining adequate calcium and vitamin D levels. [6]

Long-term exposure to high fluoride levels in drinking water may lead to disruptions in calcium and vitamin D metabolism, [7] which can impair maternal health. In Karachi, Sindh, Pakistan, drinking water fluoride levels vary, and understanding the effect of this variance on calcium and vitamin D levels in pregnant women is critical for public health. The present study aims to evaluate the association between fluoride levels in drinking water and serum calcium and vitamin D concentrations in pregnant women attending tertiary care hospitals in Karachi. This study is intended to inform healthcare policies on safe drinking water and emphasize the need for maternal nutrient monitoring, particularly in regions with high fluoride exposure.

NEED FOR THE STUDY:

This study seeks to evaluate the impact of fluoride concentrations in drinking water on calcium and vitamin D levels in pregnant women. While excessive fluoride exposure is known to disrupt mineral metabolism, its specific effects on calcium and vitamin D levels in pregnant women have not been widely studied, particularly in regions with variable fluoride levels in drinking water. [8] Current research lacks sufficient data on the relationship between fluoride intake and critical maternal nutrients like calcium and vitamin D, both essential for maintaining maternal health and fetal development. [9] This study aims to bridge this knowledge gap by assessing fluoride levels in drinking water, urine, and serum of pregnant women, and correlating these with calcium and vitamin D concentrations. By providing insights into fluoride's potential effects on maternal nutrient levels, the study will help inform health policies focused on mitigating fluoride exposure and optimizing calcium and vitamin D supplementation for pregnant women in high-fluoride regions. Additionally, this research will contribute to raising awareness regarding the implications of high fluoride exposure on maternal calcium and vitamin D status.

OBJECTIVES

- To assess the concentration of fluoride in drinking water from the patients' local areas, and measure serum and urine fluoride levels in pregnant women within one month of delivery.
- To estimate vitamin D and serum calcium levels among pregnant women close to delivery from both the areas
- To analyze the relationship between varying fluoride levels in drinking water and vitamin Study Area and Population
- This study was conducted across various tertiary care hospitals in Karachi, Sindh, Pakistan, an urban area with documented high levels of fluoride in drinking water. For comparison, water samples were also collected from Faisalabad, where fluoride levels are significantly lower. A total of 40 water samples were collected: 20 from Karachi, representing high fluoride exposure, and 20 from Faisalabad, representing low fluoride exposure.

MATERIAL & METHODS

This is a comparative cross-sectional study focusing on pregnant women attending tertiary care hospitals in Karachi, who were recruited during their prenatal visits approximately one month before their delivery date. Only pregnant women aged 18-45 years with uncomplicated pregnancies, no prior history of hypercalcemia, renal issues, or serious pregnancy complications were included. Women with multi-fetal pregnancies, smoking habits, or major health complications were excluded from the study. 20 Water samples were collected from the drinking water sources used by the participants of both the areas. Samples were stored at room temperature and analyzed within 24 hours to measure fluoride concentration. Then 180 participant samples 90 from each level of utilization of fluoride from venous blood for fluoride, calcium and vitamin D and urine samples were obtained for fluoride concentration from each participant. Blood was collected in serum separation vacutainers (SSV) and urine in sterile containers. Blood samples were centrifuged and stored at -20°C for further biochemical analysis. Fluoride concentrations in drinking water, serum, and urine were analyzed using a fluoride ion-selective electrode (Orion™ Fluoride Electrode, Thermo Fisher Scientific) following American Public Health Association (APHA) guidelines. Calibration was conducted daily using standard fluoride solutions (0.01 to 10 ppm) in the presence of a total ionic strength adjustment buffer (TISAB III). The primary biochemical parameters assessed were serum calcium and vitamin D. Blood samples were analyzed using the following procedures: Measured using a Roche Cobas e601 immunoassay analyzer. All samples were kept at a constant temperature of 2°C, monitored using a calibrated thermometer, and all assays were processed according to the manufacturer's specifications. Informed consent was obtained from each participant, and the study protocol was approved by the institutional ethics committee. Data on fluoride levels, vitamin D, and serum calcium were compared between the two groups (high fluoride and low fluoride exposure) using appropriate statistical methods to examine the potential impact of fluoride on calcium and vitamin D status in pregnant women. This study aims to provide insights into the effect of fluoride in drinking water on calcium and vitamin D metabolism among pregnant women in Karachi, supporting evidence-based health interventions for women in high-fluoride areas. D and serum calcium levels among pregnant women.

The study involved a total of 180 pregnant women, divided equally between a high-fluoride exposure group from Karachi and a low-fluoride exposure group from Faisalabad, each consisting of 90 participants. The average age of the participants was approximately 29.5 years, with a standard deviation (SD) of 5.3 years. In the high-fluoride group, the mean age was 30.1 years (SD = 5.2), while in the low-fluoride group, it was slightly lower at 28.9 years (SD = 5.4). Age-wise, the largest category was 26–35 years, making up 48.9% of the total sample, evenly distributed between the two groups. For educational background, 51.1% had completed secondary education, with 22.2% having tertiary education. Dietary intake patterns also differed slightly between the two areas; 56.7% of the participants relied on dietary sources alone for calcium and vitamin D, while 43.3% used both dietary and supplementary sources. In the high-fluoride area, 44.4% of participants used supplements, whereas in the low-fluoride group, this figure was 42.2%. Which is shown in Table 1

Table 1: Socio-Demographic Characteristics of Study Participants:

Characteristic	**Total (N=180)**	**High Fluoride Area (Karachi, N=90)**	**Low Fluoride Area (Faisalabad, N=90)**
Age (years)			
Mean ± SD	29.5 ± 5.3	30.1 ± 5.2	28.9 ± 5.4
Age Categories (years)			
18-25	56 (31.1%)	28 (31.1%)	28 (31.1%)
26-35	88 (48.9%)	44 (48.9%)	44 (48.9%)
36-45	36 (20.0%)	18 (20.0%)	18 (20.0%)
Education Level			
No Formal Education	14 (7.8%)	10 (11.1%)	4 (4.4%)
Primary	34 (18.9%)	20 (22.2%)	14 (15.6%)
Secondary	92 (51.1%)	40 (44.4%)	52 (57.8%)
Tertiary	40 (22.2%)	20 (22.2%)	20 (22.2%)
Calcium and Vitamin D Intake			
Dietary Intake Only	102 (56.7%)	50 (55.6%)	52 (57.8%)
Dietary and Supplementary Intake	78 (43.3%)	40 (44.4%)	38 (42.2%)

When examining fluoride levels in drinking water, significant differences were observed between the two groups. The high-fluoride group, representing Karachi, had an average drinking water fluoride concentration of 2.4 mg/L (SD = 0.5), whereas the low-fluoride group from Faisalabad had an average concentration of 0.6 mg/L (SD = 0.3). The mean serum fluoride concentration in the Karachi group was 0.6 mg/L (SD = 0.2), compared to 0.2 mg/L (SD = 0.1) in the Faisalabad group. Urine fluoride levels were also higher in the Karachi group, with an average of 1.8 mg/L (SD = 0.4), compared to 0.6 mg/L (SD = 0.3) in Faisalabad. In terms of biochemical parameters, the mean serum calcium level was lower in the high-fluoride group (8.8 mg/dL, SD = 0.6) than in the low-fluoride group (9.6 mg/dL, SD = 0.7). Vitamin D levels followed a similar trend, with the Karachi group showing an average concentration of 21.3 ng/mL (SD = 7.2) compared to 27.6 ng/mL (SD = 9.1) in Faisalabad. Which is shown in Table 2

Table 2: Fluoride, Calcium, and Vitamin D Levels in Pregnant Women

Parameter	**Total (N=180)**	**High Fluoride Area (Karachi, N=90)**	**Low Fluoride Area (Faisalabad, N=90)**
Fluoride Levels (mg/L)			
Drinking Water	1.5 ± 0.7	2.4 ± 0.5	0.6 ± 0.3
Serum Fluoride	0.4 ± 0.2	0.6 ± 0.2	0.2 ± 0.1
Urine Fluoride	1.2 ± 0.5	1.8 ± 0.4	0.6 ± 0.3
Biochemical Parameters			
Serum Calcium (mg/dL)	9.2 ± 0.8	8.8 ± 0.6	9.6 ± 0.7
Vitamin D (ng/mL)	24.5 ± 8.5	21.3 ± 7.2	27.6 ± 9.1

An analysis of the relationship between fluoride exposure and levels of calcium and vitamin D revealed notable differences between the two groups. The high-fluoride group, with an average drinking water fluoride concentration of 2.4 mg/L, had a mean serum calcium level of 8.8 mg/dL (SD = 0.6) and an average vitamin D level of 21.3 ng/mL (SD = 7.2). In contrast, the low-fluoride group, with a mean fluoride concentration of 0.6 mg/L, exhibited higher mean calcium and vitamin D levels, with serum calcium at 9.6 mg/dL (SD = 0.7) and vitamin D at 27.6 ng/mL (SD = 9.1). Statistical testing confirmed that these differences were significant, with a p-value of less than 0.05, indicating a likely association between elevated fluoride exposure and reduced levels of calcium and vitamin D in pregnant women. Which is shown in Table 3

Table 3: Association of Fluoride Levels with Serum Calcium and Vitamin D Levels

Group	**Mean Fluoride in Drinking Water (mg/L)**	**Serum Calcium (mg/dL) ± SD**	**Vitamin D (ng/mL) ± SD**
High Fluoride Area (Karachi)	2.4 ± 0.5	8.8 ± 0.6	21.3 ± 7.2
Low Fluoride (Faisalabad)	0.6 ± 0.3	9.6 ± 0.7	27.6 ± 9.1
p-value		<0.05	<0.05

DISCUSSION:

The analysis shows that the high-fluoride group (Karachi) had a significantly higher fluoride concentration in drinking water, serum, and urine compared to the low-fluoride group (Faisalabad). Correspondingly, pregnant women in high-fluoride areas had significantly lower serum calcium and vitamin D levels than those in low-fluoride areas. These findings highlight the potential impact of high fluoride levels on the calcium and vitamin D status of pregnant women, underscoring the need for further investigation and possible interventions to manage fluoride exposure and improve maternal health in high-fluoride areas. [10] This study investigates the impact of fluoride concentration in drinking water on serum calcium and vitamin D levels in pregnant women, comparing groups exposed to high fluoride levels (Karachi) and low fluoride levels (Faisalabad). The findings reveal significant differences in serum calcium and vitamin D levels, with lower values observed in women from high-fluoride regions, suggesting a potential correlation between fluoride exposure and reduced calcium and vitamin D availability during pregnancy. [11] These results contribute to the ongoing discourse about the potential metabolic effects of fluoride, particularly in vulnerable populations such as pregnant women, and align with existing research in related areas. The high fluoride concentrations in drinking water in Karachi (mean of 2.4 mg/L) were associated with reduced serum calcium levels, averaging 8.8 mg/dL compared to 9.6 mg/dL in the low-fluoride group from Faisalabad. This finding aligns with studies such as those by Amini et al. (2008), who observed similar decreases in calcium levels among individuals exposed to high fluoride levels. [12] The mechanism behind this effect may involve the interference of fluoride ions with calcium absorption and metabolism, as reported by Choubisa (2018), who found that chronic fluoride exposure disrupts bone and mineral metabolism, leading to reduced bioavailability of calcium. [13] The observed decrease in vitamin D levels in pregnant women from the high-fluoride area (21.3 ng/mL) compared to the low-fluoride area (27.6 ng/mL) supports similar findings by Wang et al. (2017). [14] Their research demonstrated that fluoride can negatively affect vitamin D metabolism, potentially through disruption of parathyroid function and subsequent calcium and vitamin D regulation pathways. [14] [15] The deficiency in vitamin D among women in fluoride-endemic areas might exacerbate the risk of skeletal abnormalities in the developing fetus, as vitamin D is essential for proper bone formation and mineralization during fetal development. [16]

In terms of socio-demographic characteristics, this study found that a significant proportion of participants in both groups relied primarily on dietary sources for calcium and vitamin D. However, despite this dietary intake, participants in high-fluoride areas still exhibited lower serum levels of these nutrients. This indicates that fluoride exposure might override dietary efforts to maintain adequate levels of calcium and vitamin D, which is consistent with findings by Basha and Madhuri (2014). [17] They found that fluoride's effect on mineral absorption might persist even with adequate nutrient intake, suggesting a need for tailored interventions in fluoride-affected areas. This study also highlighted the role of socioeconomic factors in supplement use, with a slightly higher prevalence of vitamin D and calcium supplementation in the high-fluoride group. Yet, these supplements did not significantly bridge the nutrient gap between the high and low fluoride exposure groups. [18] This suggests that, in regions with high fluoride exposure, standard supplementation might be insufficient and should be adjusted to compensate for fluoride's inhibitory effect on calcium and vitamin D levels. [19] These findings support recommendations by Hong et al. (2020) that nutritional interventions in fluoride-affected areas should consider both dietary intake and potential environmental inhibitors. [20]

Our findings contribute to a growing body of evidence that supports the need for fluoride regulation in drinking water, particularly in regions like Karachi where fluoride levels exceed the World Health Organization's recommended limits of 1.5 mg/L. Fluoride mitigation strategies, such as water filtration systems or public health initiatives to provide low-fluoride water sources, could play a critical role in improving maternal health outcomes. Furthermore, increased awareness of the impact of environmental fluoride exposure on maternal and fetal health could encourage healthcare providers to monitor and manage nutrient deficiencies among pregnant women in these areas.

CONCLUSION FUTURE RESEARCH DIRECTIONS:

This study demonstrates a clear association between high fluoride levels in drinking water and decreased calcium and vitamin D levels in pregnant women. The observed differences in nutrient levels between the high-fluoride group from Karachi and the low-fluoride group from Faisalabad highlight the potential of fluoride exposure to disrupt essential metabolic pathways, leading to nutrient deficiencies even when dietary intake is sufficient. Given the critical role of calcium and vitamin D in supporting maternal health and fetal development, these findings underscore the need for healthcare providers and policymakers to address fluoride exposure in high-risk regions.

Future studies should further explore the mechanisms by which fluoride interferes with calcium and vitamin D metabolism, focusing on the biochemical pathways involved in nutrient absorption and utilization. Research should also consider a larger, more diverse sample size and include longitudinal studies to evaluate the long-term impact of maternal fluoride exposure on offspring health. Additionally, interventional studies that test the efficacy of enhanced dietary or supplemental interventions in high-fluoride regions could offer valuable insights into effective strategies for mitigating fluoride’s adverse effects. Finally, exploring the role of other environmental or lifestyle factors, such as air pollution or dietary patterns, could provide a more comprehensive understanding of the multiple factors influencing nutrient status in pregnant women in high-fluoride areas.

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