

Research Article

# Impact of nutrient deficiencies on early childhood caries: A case-control study on Vitamin D, Calcium, and Ferritin serum levels

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## Introduction

Early Childhood Caries (ECC) is defined as the presence of one or more decayed (cavitated or noncavitated), missing teeth, or filled tooth surfaces in any primary tooth in a preschool child between birth and 71 months of age <sup>(1)</sup>. It is a significant global health concern, affecting young children on a worldwide scale <sup>(1)</sup>.

According to the World Health Organization, caries in primary teeth affect more than 530 million children under 6 years of age worldwide <sup>(2)</sup>. Numerous studies have shown that the global prevalence of ECC ranges from 23% to 90%, with specific studies reporting rates of 46.2% and 48%, respectively <sup>(3)</sup>. The high prevalence of dental caries in the primary dentition underscores the role of early dental care and preventive measures in children. ECC presents a substantial challenge both for caregivers and pediatric dentists, due to the difficulties involved in the treatment of young uncooperative patients <sup>(4)</sup>.

Optimal nutrition is crucial to the development and strengthening of dental structures, thereby enhancing the resistance of dental tissues to caries <sup>(5,6,7)</sup>. Key nutritional components include vitamin D, calcium, and ferritin <sup>(8,9)</sup>. The link between ECC and nutritional status has increasingly become a research focus due to its contentious nature. This includes debates about fundamental processes and impact orientation and the importance of context <sup>(10)</sup>.

**Abstract:** Background: Early Childhood Caries, often prompts healthcare professionals to focus on local factors, often neglecting systemic contributors. Therefore, research was designed to examine the relationships between blood levels of vitamin D, calcium, ferritin and the occurrence of Early Childhood Caries. Materials and Methods: This case-control study included a cohort of fifty Egyptian children aged 2 to 6 years who were segregated into two equal groups: the case group (Early Childhood Caries) and the control group (free from caries). Blood samples were taken from all participants to determine the levels of vitamin D, calcium, and ferritin. Results: In terms of vitamin D levels, 24% (n=6) of the control group had insufficient vitamin D levels (10 - <30 ng/mL) compared to 88% (n = 22) of the case group (p<0.001). Regarding calcium levels, the mean calcium level was slightly higher in the case group (9.62 ± 0.39) than in the control group (9.41 ± 0.59). with no significant difference (p > 0.05). The mean ferritin level was significantly higher in the control group (79.91 ± 49.48) than in the case group (27.72 ± 7.90) (p < 0.001). Conclusion: This research shows a link between vitamin D and ferritin levels and the occurrence of Early Childhood Caries. However, no such association was seen with calcium levels.

**Keywords:** Childhood Caries, Vitamin D, Calcium, Ferritin

Furthermore, there is evidence suggesting that nutrient deficiencies characterized by insufficient weight and growth retardation could play a role in the worsening of dental caries. However, not all researches has proven a definite connection between nutrient deficiencies and ECC. This highlights the complexity of this health issue and the need for further researches<sup>(11,12,13)</sup>. As dental healthcare providers, local determinants are often focused on when assessing dental caries, inadvertently overlooking the influence of systemic contributors. As a result, the link between some of these element deficiencies and ECC is aimed to be explored by our research.

## Materials and Methods

This was a case-control study. The size of the sample was decided based on the study of El Shiekh and Hanafy<sup>(14)</sup>. Given an effect size of 0.82, an alpha significance level of 0.05, and a study power of 80%, a total of fifty children were included in this study. The sample was evenly divided, with 25 children who had ECC (the case group) and 25 for the control group (caries free). In this case-control study, the control group was matched to the study group in terms of age and gender.

Ethical approval and informed consent: Ethical approval for this study was granted by the Research Ethics Committee of the Faculty of Dentistry, Al-Azhar University, Assiut, Egypt (AUAREC20220002-12). Prior to the dental examination, the study's objective was thoroughly explained, and written informed consent was obtained from the child's guardian. The study was conducted in accordance with the principles of the Declaration of Helsinki and adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Children who are Aged 2-6 years with or without ECC and daily brushing of the teeth are included and those with serious medical conditions such as renal failure, diabetes, and blood diseases and any disease may confounds caries occurrence and assessed nutrient parameters are excluded. Between July 2023 and December 2023, the Otolaryngology department was requested to refer all children attending for conditions like tonsillitis as these do not significantly impact dental health and in need of blood sample collection, aged 2 to 6 years, to the dental clinic. Fifty children who met the inclusion criteria were selected and divided into two groups, one with ECC and one without as the control group.

The examiner performed the dental examination was subjected to rigorous training and evaluation to ensure his competency in conducting such assessments<sup>(15,16)</sup> on a dental chair in artificial light using a plain mouth mirror and a dental probe and caries index (dmft) was recorded according to the protocols and guidelines stipulated by the World Health Organization (WHO)<sup>(17)</sup>. The Kappa value for intraexaminer calibration was 0.80.

Blood collection from children following the dental examination of both groups, they were prepared for their procedure by implementing a blood examination. A phlebotomist collected the blood, following the following steps:<sup>(18,19)</sup> The necessary tools, including a needle, syringe, and vacuum tubes, were gathered. The child's hand was lowered to make the veins more visible. A tourniquet was fastened about 4-5 finger widths above the chosen venipuncture site and the child was asked to clench their fist to make the veins stand out. The site was cleaned with 70% isopropyl alcohol for half a minute and then thoroughly dried for another half a minute. Holding the child's arm and placing a thumb below the venipuncture site, the vein was secured, and the syringe was inserted at a 30-degree angle. A 5 cm blood sample was drawn, and the tourniquet was removed before the needle was removed. The needle was gently removed, and a dry cotton ball was used to apply pressure to the site. Blood was transferred to a red tube (without any anticoagulant or preservative). The tubes were sent to the laboratory. The vacutainer tubes were kept at a temperature between 4-25°C (39-77°F). Serum analysis was performed as follows: Immunodiagnostic ELISA was used for the quantitative measurement of 25-OH-Vitamin D and calcium and ferritin levels were quantitatively assessed using an automated chemistry analyzer.

Data were analyzed using IBM SPSS software. Qualitative data are presented as frequencies and percentages in the descriptive analysis, while numerical data are presented as means and standard deviations and the medians and ranges. For statistical analysis, the Chi-Square test and Fisher Exact test were used for categorical variables, and the independent t test and the Mann–Whitney test were used for numerical variables. The level of significance was set at 5% ( $p < 0.05$ ).

**Results**

The gender distribution between the two groups was not significantly different, with boys consisting 72% of the caries-free group and 68% of the ECC group (Table 1). The age range for both groups was 3.0 - 6.0 years, with no significant difference in the mean age (Table 1). Regarding the dmft index, the mean value of dmft scores in the ECC group was (5.32±1.85).

There was a significant difference in vitamin D levels between the two groups. In the caries-free group, 76% of the children had sufficient vitamin D levels (30 - 100), while most of the ECC group (88%) had insufficient vitamin D levels (10 - <30 ng/mL). The mean vitamin D level was higher in the caries-free group (43.0 ± 18.61) compared to the ECC group (23.44 ± 6.21), (Table 2).

In terms of calcium levels, there was no statistical significant difference between the two groups. Most of the children in both groups had normal calcium levels (92% in the caries-free group and 100% in the ECC group). The mean of calcium level was slightly higher in the ECC group (9.62 ± 0.39) compared to the caries-free group (9.41 ± 0.59), (Table 3).

For ferritin levels, there was a significant difference between the two groups. Although 96% of caries-free children had normal ferritin levels, only 80% of the ECC group had normal levels. The mean ferritin level was significantly higher in the caries-free group (79.91 ± 49.48) compared to the ECC group (27.72 ± 7.90), (Table 4).

**Table 1:** Demographic distribution of subjects.

	Caries free children (n = 25)		Children with ECC (n = 25)		Test of sig.	p -value
	No.	%	No.	%		
<b>Gender</b>						
Boys	18	72.0	17	68.0	$\chi^2=0.095$	0.758
Girls	7	28.0	8	32.0		
<b>Age</b>						
Min. – Max.	3.0 – 6.0		3.0 – 6.0		t= 0.281	0.780
Mean ± SD	4.20 ± 1.08		4.12 ± 0.93			
Median	4.0		4.0			

$\chi^2$ : Chi square test, t: Student t-test, SD: Standard deviation, Min: Minimum, Max: Maximum

**Table 2:** Comparison of Vitamin D blood levels between groups.

	Caries free children (n = 25)		Children with ECC (n = 25)		Test of sig.	p - value
	No.	%	No.	%		
<b>Vitamin D (ng/mL)</b>						
Insufficient (10 - <30)	6	24.0	22	88.0	$\chi^2=20.779^*$	<0.001*
Sufficient (30 – 100)	19	76.0	3	12.0		
Min. – Max.	23.0 – 89.0		13.0 – 35.0		Z= 4.950*	<0.001*
Mean ± SD	43.0 ± 18.61		23.44 ± 6.21			
Median	36.0		23.60			

$\chi^2$ : Chi square test, Z: Z for Mann Whitney test, \*: Statistically significant at  $p \leq 0.05$

**Table (3):** Comparison of calcium blood level between groups.

	Caries free children (n = 25)		Children with ECC (n = 25)		Test of sig.	p-value
	No.	%	No.	%		
<b>Calcium (mg/dL)</b>						
<b>Normal (8.1-10.4)</b>	23	92.0	25	100.0	$\chi^2= 2.083$	<sup>FE</sup> p= 0.490
<b>Abnormal (&lt; 8.1)</b>	2	8.0	0	0.0		
<b>Min. – Max.</b>	8.40 – 10.80		8.80 – 10.10		t =1.512	0.137
<b>Mean ± SD</b>	9.41 ± 0.59		9.62 ± 0.39			
<b>Median</b>	9.30		9.70			

$\chi^2$ : Chi square test, t: Student t-test, FE: Fisher Exact test

**Table (4):** Comparison between two studied groups according to ferritin level in blood.

	Caries free children (n = 25)		Children with ECC (n = 25)		Test of sig.	p-value
	No.	%	No.	%		
<b>Ferritin (ng/mL)</b>						
<b>Normal (7-140)</b>	24	96.0	20	80.0	$\chi^2= 3.030$	<sup>FE</sup> p= 0.189
<b>Abnormal (&lt; 7)</b>	1	4.0	5	20.0		
<b>Min. – Max.</b>	9.40 – 209.0		17.30 – 39.80		t= 5.207*	<0.001*
<b>Mean ± SD</b>	79.91 ± 49.48		27.72 ± 7.90			
<b>Median</b>	69.0		27.0			

$\chi^2$ : Chi square test, FE: Fisher Exact test, t: Student t-test, \*: Statistically significant at  $p \leq 0.05$

## Discussion

In pediatric dentistry, dental caries poses a significant challenge for both caregivers and practitioners, especially when dealing with uncooperative children. Local factors of dental caries are often focused on by dental professionals, with the impact of systemic factors inadvertently overlooked. Optimal level of nutrients is crucial for the maturation and strengthening of dental structures, thus increasing the resistance of dental tissues to caries. The key nutrients include vitamin D, calcium, and ferritin.

The role of vitamin D in early childhood caries: this investigation revealed notable disparities in serum vitamin D concentrations between the two groups, showing a correlation between vitamin D insufficiency and the presence of ECC. These findings are consistent with those of Williams et al. (20), Chen et al. (21), and Schroth et al. (22). These consistent results can be attributed to vitamin D disruptions during tooth development that lead to dentin and enamel defects (23), thus increasing the susceptibility to caries. However, children with ECC often suffer persistent pain due to severe dental problems, which may alter their eating habits and result in food avoidance, many children today are increasingly engaging in video games rather than participating in outdoor sports, resulting in reduced exposure to sunlight. This lack of sunlight exposure is crucial as it plays a significant role in the synthesis of vitamin D, leading to potential deficiencies in the body, thus contributing to nutritional deficiencies.

In contrast, Kim et al. (24) and Navarro et al. (25) did not find a correlation between serum vitamin D deficiency and ECC. Consequently, they did not advocate for vitamin D supplementation as a preventive strategy against ECC. The disagreement between the studies could be attributed to several factors: Differences in study design, sample size, and population characteristics can significantly impact the results. Variations in the methods used to measure serum vitamin D levels and dental caries of permanent, primary and mixed dentition can lead to discrepancies. The presence of confounding factors such as socioeconomic status, genetic predispositions, and other health conditions might influence the outcomes. Studies that do not adequately control these variables might report different findings. Differences in geographical location and environmental factors, such as sunlight exposure, can affect vitamin D levels.

Populations in areas with limited sunlight exposure might have higher rates of vitamin D deficiency, influencing the study outcomes.

#### The Role of Calcium in Early Childhood Caries

In this investigation, there was no statistical significant difference in serum calcium concentration between children with ECC and those without ECC. Most of the children, regardless of whether they had ECC or were caries-free, showed normal calcium levels. This finding is consistent with the findings of Marques and Messer<sup>(26)</sup> and Rugg-Gunn et al.<sup>(27)</sup> who also reported no correlation between calcium level and dental caries in children.

However, contrasting studies by Jha et al.<sup>(28)</sup> and Schroth et al.<sup>(29)</sup> found a substantial disparity in calcium concentrations among children with ECC compared to those without caries. Despite these different results, the findings of the present study suggested that low vitamin D levels could be a contributing factor in the occurrence of caries in the early childhood dental caries group, despite normal blood calcium level. Vitamin D is essential for the deposition of calcium in teeth and bones. Therefore, normal blood calcium levels alone are insufficient without adequate vitamin D levels. It is also plausible that other factors could contribute to the occurrence of caries in this group<sup>(30)</sup>.

#### The Role of Ferritin in Early Childhood Caries

In this research, although the ferritin serum concentrations of children with and without ECC were within normal limits, a notable significant difference was detected in the average ferritin levels between both groups. This difference can be attributed to the broad range of normal ferritin levels in the blood. Children with ECC exhibited exceptionally low normal values, while caries-free children displayed high normal values. This finding aligns with the conclusions drawn by Jha et al.<sup>(28)</sup>, Shaoul et al.<sup>(31)</sup>, and Robert et al.<sup>(32)</sup> who found that children with ECC had significantly lower ferritin levels.

This case-control study had some limitations. The findings may not be generalizable to other populations or settings due to it was conducted on Egyptian children. Additionally, there may be confounding factors, such as socio-economic status and caregiver education levels, that were not accounted for in the study. It is also important to note that case-control studies report associations, not causality.

#### Conclusion

In summary, this study shows a link between vitamin D and ferritin levels and the occurrence of ECC, which needs further investigation. However, no such link was seen with calcium levels. These findings could potentially guide the development of preventive strategies for ECC, such as vitamin D supplementation or iron status monitoring. However, more research is needed to confirm these associations and understand the underlying mechanisms. It is also important to note that these are statistical associations and do not necessarily imply causation. Other factors not measured in this study could also contribute to the observed differences.

#### Conflict of interest:

The authors have no conflicts of interest to declare.

#### Author Contributions

Shaimaa Eldeeb and Mohamed Taha Elfezary: The authors of the concepts and critiqued the manuscript. Ahmed Safaa Waly: Gathered data, spearheaded the writing process, and reviewed the manuscript. Mohamed Elsayed Moteea and Mohammad Said Abu Samadah: Participated in the drafting of the manuscript. All named authors have made substantial contributions to the research, reviewed the manuscript, and consented to its submission.

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**تأثير نقص العناصر الغذائية على تسوس الأسنان في مرحلة الطفولة المبكرة: دراسة مقارنة لمستويات فيتامين د والكالسيوم والفيريتين في المصل**  
**محمد طه الفزاري، شيماء الديب، محمد السيد مطيع، محمد سعيد ابوصمادة، أحمد صفاء والي**  
**المستخلص:**

الخلفية: غالبًا ما يدفع تسوس الأسنان في مرحلة الطفولة المبكرة المتخصصين في الرعاية الصحية إلى التركيز على المؤثرات الموضعية، وغالبًا ما يهملون المؤثرات الجهازية. الأهداف: صُمم البحث لفحص العلاقات بين مستويات فيتامين د والكالسيوم والفيريتين في الدم وانتشار تسوس الأسنان في مرحلة الطفولة المبكرة. المواد والطرق: شملت دراسة الحالات والشواهد هذه مجموعة من الأطفال المصريين الذين تتراوح أعمارهم بين ٢ إلى ٦ سنوات. ضمت المجموعة خمسين طفلًا تم تقسيمهم إلى مجموعتين متساويتين: مجموعة الحالات لديهم تسوس ومجموعة الضبط (خالية من التسوس). تم أخذ عينات دم من جميع المشاركين لتحديد مستويات فيتامين د والكالسيوم والفيريتين. النتائج: من حيث مستويات فيتامين د، كان لدى ٢٤٪ (عدد = ٦) من المجموعة الخالية من التسوس مستويات فيتامين د غير كافية (١٠ - >٣٠ نانوغرام / مل) مقارنة بـ ٨٨٪ (عدد = ٢٢) من مجموعة تسوس اسنان الاطفال المبكر (مستوى الدلالة >٠,٠٠١). فيما يتعلق بمستويات الكالسيوم، كان متوسط مستوى الكالسيوم أعلى قليلاً في مجموعة تسوس اسنان الاطفال المبكر (٩,٦٢ ± ٠,٣٩) منه في المجموعة الخالية من التسوس (٩,٤١ ± ٠,٥٩). مع عدم وجود فرق كبير (مستوى الدلالة <٠,٠٥). كان متوسط مستوى الفيريتين أعلى بكثير في المجموعة الخالية من التسوس (٧٩,٩١ ± ٤٩,٤٨) منه في مجموعة تسوس اسنان الاطفال المبكر (٢٧,٧٢ ± ٧,٩٠) (مستوى الدلالة >٠,٠٠١). الاستنتاجات: يظهر هذا البحث وجود صلة بين فيتامين د ومستويات الفيريتين وظهور تسوس اسنان الاطفال المبكر. ومع ذلك، لم يُلاحظ أي ارتباط من هذا القبيل بمستويات الكالسيوم.