Assessment of Vitamin B$_{12}$ and Its Correlation with Dental Caries and Gingival Diseases in 10- to 14-year-old Children: A Cross-sectional Study

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ABSTRACT

Aim: To assess the level of vitamin B$_{12}$ and correlate it with dental caries [decayed, missing, and filled permanent teeth (DMFT) score] and gingival diseases [plaque index (PI) and gingival index (GI)].

Design: Healthy children according to the inclusion criteria were selected by the computerized randomization method from a school to assess the vitamin B$_{12}$ levels using Centaur/Versace machine.

Materials and methods: Blood samples were collected to assess vitamin B$_{12}$ levels using automated analyzer. Oral examination was done by a single calibrated dentist. A thorough oral examination was carried out and the DMFT, PI, and GI scores of all the children were recorded and assessed. Data were analyzed using Karl Pearson’s correlation test.

Results: Vitamin B$_{12}$ levels were deficient in 64% of the children. In boys, vitamin B$_{12}$ deficiency was found in about 76.2%, whereas, in girls it was 57.1%, which was not statistically significant. The vitamin B$_{12}$ deficient children showed a significantly high DMFT scores than the children with normal vitamin B$_{12}$ levels. The Pearson’s correlation was –0.614 for DMFT, PI value –0.663, and GI value of –0.477. The negative correlation stated that there was a reverse relation between these indices and vitamin B$_{12}$.

Conclusion: In children with systemic vitamin B$_{12}$ deficiency, there is increased dental caries prevalence and associated gingival problems.

Keywords: Dental caries, Oral health, Vitamin B$_{12}$.

INTRODUCTION

Vitamin B$_{12}$ is one of the important micronutrients for brain development and function. The developing brain was more sensitive to the deficiency of this micronutrient than the mature brain. Fetal requirements are obtained by active transport through the placenta. Vitamin B$_{12}$ (cobalamin) deficiency is common in Indians; largely owing to vegetarianism.

Micronutrient deficiency is a serious childhood problem in developing countries. Deficiencies of vitamins A, B$_{12}$, iron, folic acid, and zinc are preventable causes of poor child growth and school performance. Evidence regarding B$_{12}$ deficiency, its incidence is unknown in India, however, recent studies suggests that it is commoner than thought. Large number of cases were reported with neurological manifestations. Studies by Wadia et al$^3$ found vitamin B$_{12}$ deficiency (below 200 pg/mL) in 0.88%, folic acid deficiency in 1.1% (below 3 ng/mL), deficiency of both in 2.4% patient, and 3.8% had levels near the lower limit of normal.$^3$ It is common in India, owing to cultural and religious beliefs, low intake of animal source food, and strict vegetarianism.$^1,4,5$

The consequences of cobalamin deficiency include poor growth, megaloblastic anemia, neurological manifestations include mood changes and altered sensory, and motor and cognitive functions.$^6$

For the pediatric dentists, it is a matter of concern because oral health is the reflection of the body. Healthy teeth and gums provide a healthy body. Poor oral health has its effects to various organ systems, during pregnancy causing prematurity to long-term effects in off-springs, can be a risk factor to heart diseases. There are certain essential nutrients, micro- and macro-nutrients which are required for overall development of an individual.$^7,8$

Vitamin B$_{12}$ is found as cobalamin only in animal products. Many poor population or those who avoid animal products for religion or other reasons consume little or no Vitamin B$_{12}$. Low serum B$_{12}$ concentration is associated with a higher risk of harm to short-term memory, cognitive function, and higher risk of megaloblastic anemia.$^2,9-11$
Cobalamin deficiency is inadequate to support normal metabolic function. Inadequate intakes of nutrients, e.g., riboflavin, copper, vitamin D, and vitamin B12, are associated with increased caries experience.

Thus, in this study, an attempt is made to assess the level of vitamin B12 and correlate it with dental caries and gingival diseases.

MATERIALS AND METHODS

The protocol of the present study was approved by the ethical committee and scientific review board of the university. The study comprised of 42 children in the age group of 10 to 14 years (n = 21 girls and n = 21 boys). The children were selected randomly according to the inclusion criteria. Children who were healthy and compliant were selected. Children with any systemic illness or problems excluded from the study. Also, the patients not willing to accept proposed treatment plan and/or participate in the study were not included. Informed written consent was obtained from all the parents of children participating in the study. Assent was obtained from all the children participating in the study. After recording the preliminary information, clinical examination was carried out.

METHODOLOGY

All the adolescent children between the age group of 10 to 14 years (21 girls and 21 boys) that fit into the study were selected by computerized randomization method and screened for vitamin B12 deficiency. Blood samples were collected by pathologist. Vitamin B12 levels were assessed by Centaur/Versace machine and hemoglobin levels by BEKMANN and COULTER LH 500 automated hematology analyzer.

Sample Collection and Storage

Serum

A serum separator tube was used and 3cc blood samples were allowed to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 gm. Freshly prepared assay serum was immediately stored in aliquot at –20°C for later use. Repeated freeze/thaw cycles were avoided.

Plasma

Plasma was collected using Ethylenediaminetetraacetic acid or heparin as an anticoagulant. Samples were centrifuged for 15 minutes at 1000 gm at 2 to 8°C within 30 minutes of collection. Plasma and assay were removed immediately or samples were stored in aliquot at –20°C for later use. Repeated freeze/thaw cycles were avoided.

Oral Examination

Oral evaluation was done for all the children selected for the study. Before doing the oral examination, diet history and other relevant details if patient is under any vitamin or calcium supplements was noted.

Oral examination of all children, along with clinical examination of teeth and periodontal tissues were done by the same calibrated dentist. The visual examination was done under standard dental chair and light. Intraoral illumination was provided by a headlamp with the aid of an ordinary mouth mirror. A dental explorer was used for caries detection and gingival examination. All the teeth surfaces were dried and visualized. Scores for decayed, missing, and filled permanent teeth (DMFT) was done to assess the caries prevalence according to World Health Organization criteria.

Gingival health and oral hygiene scores were assessed using the gingival index (GI) (Löe and Silness, 1963) and plaque index (PI) (Silness and Löe, 1964). The GI scores are as follows: 0 = absence of inflammation/normal gingiva; 1 = mild inflammation, slight change in color, slight edema, and no bleeding on probing; 2 = moderate inflammation, moderate glazing, redness, edema, and hypertrophy; bleeding on probing; 3 = severe inflammation, marked redness, and hypertrophy ulceration and tendency to spontaneous bleeding. The PI scores are as follows: 0 = no plaque; 1 = a film of plaque adhering to the free gingival margin and adjacent area of the tooth. It can be seen by using a probe on the tooth surface; 2 = moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye; 3 = abundance of soft matter within the gingival pocket or on the tooth and gingival margin. The dental examination was done by one examiner only. The data were statistically analyzed by using Statistical Package for the Social Sciences 18 software. Karl Pearson’s correlation method was used to find out the relation between the DMFT, GI, and PI

Results: There were equal number of girls and boys in the present study. The case history revealed all the children had similar oral hygiene practices and did not have any other systemic problems or were not under any medication. The vitamin B12 levels were deficient in 64% of the children (Graph 1). In boys, vitamin B12 deficiency was found in about 76.2%, whereas, in girls it was 57.1% (Graph 1) which was not statistically significant. In the diet and gender distribution, 52.4% boys had a vegetarian diet, whereas only 19% girls had a vegetarian diet (Graph 2). The mean DMFT score was 3.810, PI score was 1.376, and GI score was 0.914. The vitamin B12 deficient
children showed a significantly high DMFT scores than the children with normal vitamin B₁₂ levels. The Pearson’s correlation being -0.614 for DMFT, PI value of -0.663, and GI value of –0.477. In Tables 1 to 3, the diet and dental caries relation also showed that the dental caries was higher among the vegetarian diet as compared to the non-vegetarian diet (Tables 4 and 5). The negative correlation stated that there was a reverse relation between these indices and vitamin B₁₂ stating that when the vitamin B₁₂ is deficient, there increase in dental caries and gingival problems.

Table 1: Correlation between dental caries and vitamin B₁₂ levels using Karl Pearson’s correlation

| DMFT          | Vitamin B₁₂ | Pearson’s correlation | Sig. (2-tailed) | N  | 42 |
|---------------|-------------|-----------------------|-----------------|----|----|
| Vitamin B₁₂   | Pearson’s correlation | −0.614** | 0.000 | 42 | 42 |
| Sig. (2-tailed)|              | 0.001                 |                 |    |    |

**Correlation is significant at the 0.01 level (2-tailed)

Table 2: Correlation between plaque index and vitamin B₁₂ levels using Karl Pearson’s correlation

| PI            | Vitamin B₁₂ | Pearson’s correlation | Sig. (2-tailed) | N  | 42 |
|---------------|-------------|-----------------------|-----------------|----|----|
| Vitamin B₁₂   | Pearson’s correlation | −0.663** | 0.000 | 42 | 42 |
| Sig. (2-tailed)|              | 0.000                 |                 |    |    |

**Correlation is significant at the 0.01 level (2-tailed)

Table 3: Correlation between gingival index and vitamin B₁₂ levels using Karl Pearson’s correlation

| GI            | Vitamin B₁₂ | Pearson’s correlation | Sig. (2-tailed) | N  | 42 |
|---------------|-------------|-----------------------|-----------------|----|----|
| Vitamin B₁₂   | Pearson’s correlation | −0.477** | 0.001 | 42 | 42 |
| Sig. (2-tailed)|              | 0.001                 |                 |    |    |

**Correlation is significant at the 0.01 level (2-tailed)

Table 4: Correlation between diet, B₁₂ deficiency, and DMFT scores

| Diet          | Vegetarian | Non-vegetarian | Mean DMFT score |
|---------------|------------|----------------|-----------------|
| Count         | 10         | 18             | 5.4             |
| % within diet | 66.7%      | 23.8%          | 33.3%           |
| % of total    | 23.8%      | 42.9%          | 100.0%          |
| Count         | 5          | 9              | 14              |
| % within diet | 66.7%      | 66.7%          | 100.0%          |
| % of total    | 33.3%      | 33.3%          | 100.0%          |
| Average DMFT scores | 4.5 | 3.04 |

Table 5: Correlation between diet and vitamin B₁₂ levels using Karl Pearson’s correlation

| Vitamin B₁₂ | Deficient | Normal | Total | Average DMFT scores |
|-------------|-----------|--------|-------|---------------------|
| Diet        | Count     | % within diet | % of total | Count | % within diet | % of total | Count | % within diet | % of total |
| Vegetarian  | 10        | 66.7%   | 23.8% | 18     | 66.7%   | 42.9%     | 28    | 66.7%   | 33.3% |
| Non-vegetarian | 5        | 33.3%   | 76.2% | 9      | 33.3%   | 66.7%     | 14    | 33.3%   | 66.7% |
DISCUSSION

Many elements in trace amounts are known to contribute to the oral hygiene and health. Vitamin B₁₂ is an essential element which is not produced by the body. It is mainly found exclusively in animal products, such as meat, eggs, fish, minute quantities in milk, soy, etc. It is important in the early growth of the child as it affects brain development and function, memory, reasoning, attention, metabolism, formation of RBC’s, and oral hygiene as stated by various authors. Inadequate intakes of nutrients (e.g., riboflavin, copper, vitamin D, and vitamin B₁₂) associated with increased caries experience. Adequate intake vs inadequate or high adequate intakes of nutrients (e.g., vitamin B₁₂ and vitamin C) were associated with decreased caries experience.

Vitamin B₁₂ is one of the most common deficiencies in the Indian population, however, most often undetected and found as an accidental finding. The relationship between vitamin B₁₂ deficiency and oral health still remains unclear. The available medical literature does not show any study performed with such a correlation. Since various studies have stated that the oral conditions differ from geographic and social conditions. The children going to the same school and having similar dental care habits and characteristics were selected for this study. Also other systemic illnesses or any calcium supplements or medications that would hamper the oral hygiene were taken into consideration to eliminate any false results or bias. The method used for vitamin B₁₂ assessment is the most commonly used and also standard as stated in various studies.

The DMFT/decayed, missing and filled surface indices are the most commonly used tools to determine the caries prevalence and health status of the population. The GI and PI scores are the gold standards for examining the oral hygiene status of the subjects. In this study, the overall effect of vitamin B₁₂ deficiency was measured together on the teeth and gingiva.

There is not much data available in this regard. Marshall found that inadequate intakes of low adequate or high adequate intakes of nutrients (e.g., riboflavin, copper, vitamin D, and vitamin B₁₂) were associated with increased caries experience and low adequate intakes of inadequate or high adequate intakes of nutrients (e.g., vitamin B₁₂ and vitamin C) were associated with decreased caries experience.

Pontes et al also found presence of oral signs and symptoms, including glossitis, angular cheilitis, recurrent oral ulcer, oral candidiasis, diffuse erythematous mucositis, and pale oral mucosa in subjects with cobalamin deficiency offering the dentist an opportunity to participate in the diagnosis of this condition. Supplementation with vitamin B₁₂ will improve the gingival health and oral hygiene of children with deficiency, the present study was only a cross-sectional and the changes in the oral health could not be demonstrated, which will be further explored in further studies. Also an in-depth salivary analysis to rule out any other causes with a larger sample size will further validate the results of the present study.

The presence of statistically significant results with a reverse relation makes a good evidence and logic for the association between them. As pediatric dentists, we must be aware of the possible deficiency taking into consideration the history, clinical signs, and also investigations whenever required. This will prevent the further progress of the disease and any irreversible damage to the neural and cognitive function.

CONCLUSION

Vitamin B₁₂ deficiency may cause an increase in prevalence of dental caries and gingival diseases in children. As pediatric dentist, our role is not just giving dental care to the child but it also includes the overall health care, behavior assessment which will provide us with the sign and symptoms of any deficiency or abnormality in child and with timely referral to pediatrician and concern of the dentist, we can prevent any permanent damage to the children.

REFERENCES

1. Bhate V, Deshpande S, Bhat D, Joshi N, Ladkat R, Wate S, Fall C, de Jager CA, Refsum H, Yajnik C. Vitamin B₁₂ status of pregnant Indian women and cognitive function in their 9-year-old children. Food Nutr Bull 2008 Dec; 29(4):249-254.
2. Uchendu FN. Micronutrient malnutrition, a tragedy to childhood growth and education. Glob J Med Res 2011 May;11(1):27-34.
3. Wadia RS, Bandishiti S, Kharche M. B₁₂ and folate deficiency: incidence and clinical features. Neurol India 2000 Dec;48(4):302-304.
4. Louwman MW, van Dusseldorp M, van de Vijver FJ, Thomas CM, Schneeke J, Ueland PM, Refsum H, van Staveren WA. Signs of impaired cognitive function in adolescents with marginal cobalamin status. Am J Clin Nutr 2000 Sep;72(3):762-769.
5. McLean ED, Allen LH, Neumann CG, Peerson JM, Siedmann JH, Murphy SP, Bwibo NO, Demment MW. Low plasma vitamin B₁₂ in Kenyan school children is highly prevalent and improved by supplemental animal source foods. J Nutr 2002 Mar;132(3):676-682.
6. Rogers LM, Boy E, Miller JW, Green R, Sabel JC, Allen LH. High prevalence of cobalamin deficiency in Guatemalan schoolchildren: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. Am J Clin Nutr 2003 Feb;77(2):433-440.
7. Navia JM. Prevention of dental caries: agents which increase tooth resistance to dental caries. Int Dent J 1972 Dec;22(4):427-440.
8. Babaji P, Shashikaran NN, Subba Reddy VV. Comparative evaluation of trace elements and residual bacterial content of different brands of bottled water. J Indian Soc Pedod Prev Dent 2004 Oct-Dec;22(4):201-204.

9. Arsenault JE, Mora-Plazas M, Forero Y, López-Arana S, Marin C, Baylin A, Villamor E. Provision of a school snack is associated with vitamin B-12 status, linear growth, and morbidity in children from Bogota, Columbia. J Nutr 2009 Sep;139(9):1744-1750.

10. Nyaradi A, Li J, Hickling S, Foster J, Oddy WH. The role of nutrition in children’s neurocognitive development, from pregnancy through childhood. Front Hum Neurosci 2003 Mar;26;7:97.

11. Pontes HA, Neto NC, Ferreira KB, Fonseca FP, Vallinoto GM, Pontes FS, Pinto Ddos S Jr. Oral manifestations of vitamin B12 deficiency: a case report. J Can Den Assoc 2009 Sep;75(7):533-537.

12. Black MM. Micronutrient deficiencies and cognitive functioning. J Nutr 2003 Nov;133(11 Suppl 2):3927S-3931S.

13. Marshall TA, Levy SM, Broffitt B, Warren JJ, Eichenberger-Gilmore JM, Burns TL, Stumbo PJ. Dental caries and beverage consumption in young children. Pediatrics 2003 Sep;112(3 Pt 1):e184-e191.

14. Atasoy HB, Ulusoy ZI. The relationship between zinc deficiency and children’s oral health. Pediatr Dent 2012 Sep-Oct;34(5):383-386.

15. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev 2008 May;66(5):250-255.

16. Sheetal A, Hiremath VK, Patil AG, Sajjasnetty S, Kumar SR. Malnutrition and its oral outcome – a review. J Clin Diagn Res 2013 Jan;7(1):178-180.