Prevalence of serum cobalamin and folate deficiency among children aged 6–59 months: A hospital-based cross-sectional study from Northern India

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ABSTRACT

Context: Cobalamin and folate are essential for the synthesis of nucleic acids and in the maintenance of myelin. They are required during the period of the rapid growth of infancy and childhood. Their deficiency may result in nutritional anemia and neurological manifestations. There is paucity of literature regarding the prevalence of cobalamin and folate deficiency among North Indian children aged 6–59 months.

Aim: The aim of this study was to estimate the prevalence of serum cobalamin and folate deficiency among children aged 6–59 months, attending a secondary care hospital.

Settings and Design: Children were recruited from pediatrics OPD of the sub-district hospital (SDH), Ballabgarh, Haryana, through systematic random sampling. Hemoglobin was measured by an automated analyzer. Serum cobalamin and serum folate were estimated using enhanced chemiluminescence based immunoassay.

Results: A total of 420 children were recruited, of which 392 provided a blood specimen. Prevalence of cobalamin, folate deficiency, and anemia were 22.3% (95% CI: 18.3–26.7), 10.9% (95% CI: 8.2–14.6), and 81.9% (95% CI 77.7–85.4), respectively. The proportion of children with cobalamin deficiency who had anemia was 97.7% compared to 95.7% among those with no cobalamin deficiency (P = 0.396). Similarly, the proportion of children with folate deficiency who had anemia was 95.2% compared to 96.2% among those with no folate deficiency (P = 0.765).

Conclusion: We found that almost one in five children aged 6–59 were deficient in cobalamin, and one in ten were deficient in folate.

Keywords: 6–59 months, cobalamin deficiency, folate deficiency, micronutrient deficiency, nutritional anemia, under 5 aged children

Introduction

Cobalamin and folate are required during the period of the rapid growth of infancy and childhood.[11] Together or individually, their deficiency during early childhood can lead to poor growth, proneness to infection, irreversible neurological damage to the developing brain, impairment in memory, compromised cognition, poor school performance, reduced work capacity, and

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stunting. Their deficiency can have nonspecific manifestations like delaying the development and growth, weakness, and irritability. Permanent neurologic damage may occur if left untreated. Different kinds of irreversible neurologic damage among children, e.g., developmental regression, microcephaly, apathy, hypotonia, hypokinesia, etc., have been reported in the literature. Hence, early identification and treatment of cobalamin and folate deficiency by physicians, at secondary and primary level, is required to maintain normal growth among under-five children.

Literature has reported a widely varying prevalence of cobalamin deficiency (2.3% to 28.0%), and of folate deficiency (1.3% to 63.0%). The reason for such wide variation is not known, but it could partially be due to geographic location. Hence, we carried out this study that would be locally relevant. The objective of the study was to estimate the prevalence of serum cobalamin and folate deficiency among children aged 6–59 months attending the pediatrics OPD of the secondary level hospital, Ballabgarh, district Faridabad, Haryana.

**Subjects and Methods**

This study was carried out between June and August 2018 among children attending pediatrics OPD of a Sub-District Hospital located in Ballabgarh Block of district Faridabad, Haryana. The sample size of 420 was arrived based on the assumption of the prevalence of cobalamin deficiency among children as 30%, absolute precision of 5%, and a non-response rate of 20%. For folate deficiency, the prevalence was assumed to be 46% and the calculated sample size was 460. However, considering resource limitations, a final sample size of 420 was chosen. Through systematic random sampling, we identified every fifth child in the age group 6–59 months that reported to the pediatric OPD for the first time during the study period. The exclusion criteria were history of malignancy, immunosuppressive disorder, hemoglobinopathies, blood transfusion, intake of multivitamin or iron-folic acid syrup, and any debilitating disease requiring emergency care in the last 6 months.

Written informed consent was obtained from parent/guardian/accompanying adult. Hemoglobin along with blood indices were measured using an automated analyzer (Sysmex XS-1000i). Anemia was defined as a hemoglobin level less than 11 g/dL. Serum cobalamin and serum folate were estimated by Enhanced Chemiluminescence based Immunoassay autoanalyzer (VITROS EGiQ, Ortho Clinical Diagnostics, New Jersey, USA) using standard technique. The laboratory where these tests were done had a well-functioning EQAS program and the technicians were experienced in performing the test. The serum level to define deficiency was as per the WHO recommendations, i.e., less than 203 pg/mL for cobalamin and less than 4 ng/mL for folate.

We excluded from the final analysis those children whose parent/guardian/accompanying adult did not consent to provide a blood specimen. The analysis was done using STATA Vs 12.0 statistical software (Stata Corp. 2012. Stata Statistical Software: Release 12. College Station, TX: Stata Corp LP). The result is reported as frequencies and percentages for categorical variables, and as mean ± SD for continuous variables. Prevalence of outcome variables is reported as a proportion (n, %) with 95% confidence interval. Logistic regression analysis was performed to evaluate the odds ratio (OR) and 95% CI for children with cobalamin and folate deficiency. Variables with P < 0.25 in the univariate analyses were included in multivariate analysis. The P values of less than 0.05 were considered statistically significant. The study was approved by the Institute Ethics Committee of the All India Institute of Medical Sciences, New Delhi (vide letter no. IECPG-527/20.12.2017, RT-60/31.01.2018; dated: 12.2.2018).

**Results**

A total of 420 children were enrolled in the study, and most (n = 392, 93.3%) provided a blood specimen. Among those who provided blood specimens, serum cobalamin and serum folate level were available for 386 (98.5%) and 383 (97.7%) children, respectively. The blood specimen for the remaining children was insufficient in quantity to perform the laboratory tests.

The mean ± SD age of the children was 28.4 ± 14.2 months, and the majority (53%) of them belonged to the second and third years of life. The proportion of male children was 55.7%.

**Hemoglobin level and blood indices**

Of the 392 children, 321 (81.9%, 95% CI 77.7–85.4) had anemia. The mean hemoglobin level was 9.5 g/dL (95% CI 9.4–9.7). The median hemoglobin level was 9.7 g/dL with an inter-quartile range (IQR) of 8.5–10.7 g/dL. Among the anemic children, the most common red blood cell morphology was the microcytic normochromic cell [Table 1].

**Serum cobalamin and folate level**

The mean (SD) serum cobalamin level was 306.9 pg/mL (143.7) (95% CI: 292–321). The median cobalamin level was 267.5 pg/mL (IQR: 210–366). The prevalence of cobalamin deficiency among children was 22.3% (95% CI: 18.3–26.7). The mean (SD) folate level was 9.9 ng/mL (5.4) (95% CI: 9.4–10.5). The median folate level was 8.5 ng/mL (IQR: 5.47–13.7). The prevalence of folate deficiency was 10.9% (95% CI: 8.2–14.6). The proportion of children with cobalamin deficiency who had anemia was 97.7% compared to 95.7% among those with no cobalamin deficiency. This difference was not statistically significant (P = 0.396). Similarly, the proportion of children with folate deficiency who had anemia was 95.2% compared to that of 96.2% among those with no folate deficiency. This difference was not statistically significant (P = 0.765).

**Factors associated with serum cobalamin and folate deficiency**

In univariate analysis, cobalamin and folate deficiencies were significantly associated with female sex (crude OR: 1.77, 95% CI:
1.09–2.88, P value: 0.02). Cobalamin deficiency was significantly associated with serum folate levels (crude OR: 1.07, 95% CI: 1.02–1.12, P value: 0.003). Folate deficiency was significantly associated with female sex (crude OR: 1.94, 95% CI: 1.02–3.75, P value: 0.044) and hemoglobin levels (crude OR: 0.69, 95% CI: 0.56–0.84, P value: < 0.001) [Table 2].

In multivariate analysis, cobalamin level was significantly associated with female sex and serum folate level (adjusted OR: 2.06, 95% CI: 1.24–3.45; and adjusted OR: 1.07, 95% CI: 1.02–1.13, respectively). In multivariate analysis, serum folate deficiency was significantly associated with female sex and hemoglobin level (adjusted OR: 2.03, 95% CI: 1.04–4.04, P value: 0.041; and adjusted OR: 0.72, 95% CI: 0.58–0.88, P value: 0.002, respectively) [Table 3].

### Discussion

We estimated the prevalence of serum cobalamin and serum folate deficiency among children aged 6–59 months attending a secondary level hospital in North India. We found that one in five children was deficient in cobalamin, and one in ten children was deficient in folate.

#### Deficiency of serum cobalamin

The prevalence of cobalamin deficiency in our study was 22.3%. According to the Comprehensive National Nutrition Survey (CNNS), the prevalence of cobalamin deficiency among children aged 1–4 years in Haryana was 11.6% (95% CI: 7.5–17.5) for children aged 1–4 years in Haryana. The higher prevalence in our study could be explained by the fact that we recruited children from a hospital setting where children were likely to be suffering from some morbidity and thus would not be representative of community-dwelling children recruited in CNNS. We found only one study that was conducted among 200 children aged 6–59 months. This study reported a higher prevalence (43%, 95% CI 36.1%–49.8%) of cobalamin deficiency. Studies conducted among children in age group other than 6–59 months of age in Northern India have reported the prevalence of cobalamin deficiency ranging between 17% and 97.5%.[11,13,17,24] Studies done in other parts of India have reported a lower prevalence in the range of 2.3% to 14%.[10,22] Probable reasons for such a wide range of prevalence could be the use of different laboratory methods, different cut-off values, different geographic locations, and different age groups for estimating cobalamin levels. Five studies had used the chemiluminescence immunoassay, which was the method used by us.

We did not find any significant association between cobalamin deficiency and the presence of anemia. Since we had not measured the serum ferritin level, we are unable to say if the observed anemia was due to iron deficiency. However, RBC morphology strongly suggested that to be the case.

We found that the serum cobalamin level was associated with female sex. Studies by Ng’eno et al.[26] Kapil et al.,[13] and Chakraborty et al.[27] reported findings similar to our study, i.e., higher prevalence of cobalamin deficiency was observed among females. However, studies by Bhardwaj et al.[28] Herrán et al.,[29] and Gupta et al.[30] report findings where female sex was associated with higher mean serum cobalamin levels. Inconsistent association of cobalamin deficiency with female sex needs to be investigated for mediators and confounders.

#### Serum folate level

The prevalence of folate deficiency in our study was 10.9% which was close to the prevalence of folate deficiency of 14.6% (95% CI: 9.1–22.6) reported by Comprehensive National Nutrition Survey for Haryana.[10] The prevalence of folate deficiency has been reported in the range of 1% to 63% across various regions of India.[10‑13,18‑22,26] We did not find any other study on serum folate level in children aged 6–59 months from India. However, a community-based cross-sectional study by Gupta et al.[31] reported folate deficiency among 1.5% adolescents. Similarly, a study conducted by Kapil and Bhardwaj in Delhi reported folate deficiency of 40% among adolescents aged 11–18 years. These studies are not strictly comparable to our study. Yet our findings are within the broad range of reported prevalence rate of folate deficiency. In our study, we found a positive association between female sex and folate deficiency, which is in agreement with study by Kapil et al.[13] and Gupta et al.[31] We did not find any significant association between folate deficiency and the presence of anemia (P = 0.77). However, our study was not powered to detect the association even if it truly existed.

We observed a significant association between folate and cobalamin levels (P value: 0.003). It could be due to functional deficiency of folate secondary to cobalamin deficiency commonly known as folate trap phenomenon. Here, the observed serum folate level becomes artificially high among those who are concurrently cobalamin deficient due to the noncompletion of further steps requiring cobalamin.[31]

We found that the prevalence of anemia among children aged 6–59 months attending pediatrics OPD of a sub-district...
hospital was 82%. Our findings were higher than the figure reported by CNNS (48.3%, 95% CI: 40.3–56.3) for the state of Haryana.[16] The observed difference could have been due to differences in age group included (1–4 years), source population (community), and the method deployed for measuring hemoglobin (cyanmethemoglobin) in CNNS.[16] Majority of the children in our study had microcytic normochromic red blood cells suggesting iron deficiency. Only

| Characteristic                                                                 | Cobalamin deficiency | Serum folate deficiency |
|-------------------------------------------------------------------------------|----------------------|------------------------|
| Sex of the child                                                              | OR (95% CI)          | P          | q2    | OR (95% CI)           | P         | q2    |
| Male                                                                          | -                    | 0.02       | 0.14  | -                    | 0.044     | 0.21  |
| Female                                                                        | 1.77 (1.10-2.88)     | <0.001     | <0.001 | 1.94 (1.02-3.75)     | <0.001    | <0.001|
| Age of the child (Months)                                                     | 0.99 (0.97-1.01)     | 0.21       | 0.41  | 1.01 (0.98-1.03)      | 0.65      | 0.8   |
| Age of the mother when participant child was born                             | 0.98 (0.91-1.04)     | 0.49       | 0.68  | 1.02 (0.93-1.10)      | 0.67      | 0.8   |
| Period of gestation                                                           | Pre-Term             | <0.001     | <0.001 | -                    | 0.58      | 0.8   |
| Term                                                                          | 1 (0.46-2.44)        | 1.39 (0.47-5.98)| 0.8   |
| Birth weight*                                                                 | <2.5 kg              | 1.4 (0.86-2.29)| 0.23  |
| >2.5 kg                                                                       |                      | 0.67 (0.34-1.29)|        |
| Birth order                                                                   | 1                     | 0.53       | 0.68  | -                    | 0.64      | 0.8   |
| 2                                                                             | 0.93 (0.52-1.66)     | 0.9 (0.41-1.95) |        |
| 3                                                                             | 1.47 (0.75-2.82)     | 0.97 (0.36-2.41) |        |
| 4                                                                             | 0.52 (0.12-1.63)     | 2.27 (0.68-6.64)|        |
| 5                                                                             | 1.81 (0.46-6.15)     | 0.74 (0.04-4.23)|        |
| >5                                                                            | 1.21 (0.06-9.80)     | 0          |        |
| Exclusive breast feeding                                                      | No                   | 0.16       | 0.41  | -                    | 0.69      | 0.8   |
| Yes                                                                           | 1.96 (0.94-4.62)     | 0.73 (0.33-1.78)|        |
| Weight-for-age Z-score                                                        | Normal (z-score -2 SD to +2 SD) | - | 0.23 | 0.41 | - | 0.91 | 0.92 |
| Underweight (z-score -2 SD to -3SD)                                           | 1.68 (0.98-2.87)     | 1.04 (0.48-2.12) |        |
| Severe Underweight (z-score < -3SD)                                           | 1.19 (0.54-2.44)     | 0.89 (0.29-2.29) |        |
| Overweight (z-score > +2 SD)                                                  | 4.2 (0.16-108)       | 0          |        |
| Height-for-age Z-score                                                        | Normal (z-score -2 SD to +2 SD) | - | 0.11 | 0.41 | - | 0.92 | 0.92 |
| Stunted (z-score -2 SD to -3SD)                                               | 1.93 (1.09-3.37)     | 0.85 (0.36-1.84) |        |
| Severely stunted (z-score < -3SD)                                             | 1.54 (0.81-2.85)     | 1.08 (0.45-2.37) |        |
| Mid Upper Arm Circumference (cm)                                              | Normal (>13.5 cm)    | - | 0.7 | 0.82 | - | 0.6 | 0.8 |
| Mild (12.5-13.5)                                                              | 0.89 (0.50-1.59)     | 1.15 (0.55-2.43) |        |
| Moderate (12.5-11.5 cm)                                                       | 1.22 (0.65-2.27)     | 0.63 (0.23-1.57) |        |
| Severe (<13.5 cm)                                                             | 0.69 (0.15-2.29)     | 0.97 (0.14-3.93)|        |
| Type of family                                                                | Extended             | - | 0.31 | 0.48 | - | 0.53 | 0.8 |
| Nuclear                                                                       | 0.77 (0.47-1.26)     | 0.81 (0.41-1.55)|        |
| Socio-economic status (monthly income in ₹)[¶]                                | Upper class (≥6254)  | - | 0.83 | 0.89 | - | 0.6 | 0.8 |
| Upper middle class (3127-6253)                                                | 1.14 (0.31-4.18)     | 2.43 (0.43-18.7) |        |
| Middle class (1876-3126)                                                      | 1.09 (0.45-3.07)     | 1.88 (0.53-12.1) |        |
| Lower middle class (938-1875)                                                 | 1.62 (0.57-5.08)     | 1.83 (0.39-13.1)|        |
| Lower class (<938)                                                            | 1.03 (0.32-3.51)     | 0.78 (0.09-6.81)|        |
| Hemoglobin (g/dL)                                                             | 1.1 (0.94-1.27)      | 0.69 (0.56-0.84) | <0.001 | 0.003 |
| Serum folate (ng/mL)[^]                                                       | 1.07 (1.02-1.12)     | 0.003      | 0.039 | - | - | - |
| Serum cobalamin (pg/mL)[^]                                                    | -                    | - | - | 1 (1.00-1.00) | 0.015     | 0.1 |

OR=Odds Ratio, CI=Confidence Interval, *False discovery rate correction for multiple testing, †Total observations for birth weight were 383, ^Values are taken from modified BG Prasad scale, ¶Indian Rupee: official currency of India
seven children (1.8%) had megaloblastic anemia indicating deficiency of cobalamin/folate.

We have added evidence that there is deficiency of cobalamin and folate among under five children in India. It is emphasized that normal cobalamin and folate levels among young children are essential for their adequate physical and mental growth. Our results indicate that cobalamin and folate levels were low in the children visiting this hospital. Both these deficiencies were associated with female sex. It is critical that physicians are sensitive to these facts and screen for cobalamin and folate deficiency, among under five children, even in primary care settings also.

**Strengths**

The refusal rate for providing the blood specimen was low. Estimation of serum cobalamin and folate were performed in a laboratory with strict quality control measures in place. The laboratory technicians were well trained to perform the test. Laboratory personnel were unaware of the study objective. Widely accepted laboratory methods were used for the estimation of cobalamin and folate levels.

**Limitations**

Since ours was a hospital-based study, the observed prevalence rates cannot be extrapolated to the community at large. The study design was cross-sectional in nature. Therefore, we can only report association rather than causation. We did not measure serum ferritin levels. Therefore, no direct evidence can be provided to assert that the anemia was due to iron deficiency. Measurement of additional sensitive metabolic indicators like methylmalonic acid and homocysteine could have increased the detection of functional cobalamin and folate deficiencies.

The use of non-fasting blood specimens might have led to an underestimation of the prevalence of folate deficiency.

**Conclusion**

The prevalence of cobalamin and folate deficiency was 22.3% and 10.9%, respectively, among children aged 6–59 months who attended pediatrics OPD of a sub-district hospital in Ballabgarh Block of district Faridabad, Haryana. Both cobalamin and folate deficiencies were associated with the female sex.

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**Key Messages**

Cobalamin and folate deficiency causes anemia and impairs memory and learning in children. High prevalence of their insufficiency has been reported in school-age children in India.

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**Table 3: Multivariate analysis showing factors associated with cobalamin and folate deficiency**

| Characteristic                  | Cobalamin deficiency | Serum folate deficiency |
|--------------------------------|----------------------|-------------------------|
|                                | aOR (95% CI)         | P                       |
|                                |                      |                         |
| Sex of the child               |                      |                         |
| Male                           | -                    | -                       |
| Female                         | 2.06 (1.24-3.45)     | 0.006                   |
| Age of the child (months)      | 1 (0.98-1.02)        | 0.9                     |
| Birth weight                   |                      |                         |
| <2.5 kg                        | -                    | -                       |
| >2.5 kg                        | 1.45 (0.87-2.44)     | 0.2                     |
| Exclusive breast feeding       |                      |                         |
| No                             | -                    | -                       |
| Yes                            | 1.68 (0.78-4.05)     | 0.2                     |
| Height-for-age Z-score         |                      |                         |
| Normal (z-score -2 SD to +2 SD)| -                    | -                       |
| Stunted (z-score -2 SD to -3SD)| 2.16 (1.18-3.93)     | 0.012                   |
| Severely stunted (z-score < -3SD)| 1.65 (0.84-3.18)  | 0.14                    |
| Serum folate (ng/mL)           |                      |                         |
| Normal                         | 1.07 (1.02-1.13)     | 0.006                   |
| Hemoglobin (g/dL)              |                      |                         |
| Normal                         | -                    | -                       |
| <7 g/dL                        | -                    | -                       |
| >7 g/dL                        | 0.72 (0.58, 0.88)    | 0.002                   |
| Cobalamin (pg/mL)              |                      |                         |
| Normal                         | -                    | -                       |
| <300 pg/mL                     | -                    | -                       |
| >300 pg/mL                     | 1 (1.00, 1.00)       | 0.019                   |

aOR=Adjusted Odds Ratio, CI=Confidence Interval
In a hospital-based cross-sectional study, we have found high prevalence of their deficiency among children aged 6–59 months. Their deficiency was associated with female sex.

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**Conflicts of interest**

There are no conflicts of interest.

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