Section: Pediatrics

Original Research Article

OBSERVATIONAL STUDY ON VITAMIN B12 AND FOLATE DEFICIENCY IN ANEMIC CHILDREN ATTENDING THE TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Objective: To study the Vitamin B12 and folate deficiency in anemic children of age group 6 months to 5 years attending the tertiary care pediatric hospital.

Materials and Methods: Present study was a cross-sectional observational hospital-based study. It was done in patients admitted in Pediatric department of Deen Dayal Hospital Upadhyay Hospital, Delhi in 2015-2017 of age group 6 month to 5 years. Total 252 children were included in present study. Out of the 252 children 112 (44.5%) were female and 140 (55.56%) were male. In each group male outnumber the female except in 37-60 month. Maximum number of children 121(48%) were present in age group 13-36 month. Out of total 252 children, 184 (73.01%) were Lower lower socioeconomic class and 68(26.98%) were upper lower

Results: In our study out of the 252 children the Pure or mixed vitamin B12 deficiency was seen 95(37.7%). Pure or mixed folate deficiency was found in 37(14.7%) children. vitamin B12 deficiency 74 (29.3%) and folate deficiency 22(8.7%) alone or combination 4 (1.5%) was seen in our study.

Conclusion: The prevalence of vitamin B12 is more common in age group in 6-12 months anemic children who were on breast fed, the reason may be maternal deficiency of vitamin B12. So, there is a strong need to supplement the Vitamin B_{12} in infancy for the normal development and healthy life of children. We recommend in policy making in fortification of food to prevent nutritional anemia secondary to vitamin B_{12} and folate deficiency.

Keywords: National Family Health survey [NFHS], nutritional anemia, vitamin B_{12} and folate deficiency.

INTRODUCTION

National Family Health survey [NFHS]-3 in India shows that 7 out of every 10 children in the age group of children are severely anemic. NFHS shows the prevalence of anemia in children is 76.4 %. Studies have shown iron, folate and vitamin B 12 to be the cause of nutritional anemia. Iron deficiency is the most important of cause of anemia. Besides iron deficiency anemia, the importance of other micronutrients in causation of anemia is being realized. However, there is limited data on prevalence of vitamin B 12 deficiency amongst children in India. Vitamin B12 deficiency is associated with a spectrum of disease from asymptomatic to serious haematological, neurologic, and psychiatric manifestations and the possible risk of irreversible neurological damage despite treatment. Children with vitamin B12 deficiency present with non-specific manifestations such as weakness, lethargy, feeding difficulties, failure to thrive, and irritability. Other common findings include pallor, glossitis, vomiting, diarrhoea and icterus. Neurologic symptoms can include paresthesia, sensory deficits, hypotonia, seizures, developmental delay, developmental regression, and neuropsychiatric changes. However, it is important to note that the neurological changes associated with low vitamin B12 can occur without hematological changes.

Vitamin B12 is found as cobalamin in foods of animal origin and cannot be synthesized in human. Liver, kidney, meat, fish, salmon, crabs, oysters egg

yolk etc. are rich sources. The most frequent cause is inadequate intake. The racial, religious, ethnic, and socioeconomic heterogeneity of the people in India greatly influences their dietary habits. Well-recognized groups that are at risk of becoming vitamin B12 deficient are vegetarians and lactovegetarians. For vegetarians and lactovegetarians, the only sources of vitamin B12 are dairy products and eggs, and fortified soymilk alternatives. The most important cause of vitamin B12 deficiency in infants is maternal dietary deficiency. It is generally observed in infants breast fed by mother who are strict vegetarians.

Folate are heterocyclic compound consisting of pteroyl group conjugated with one or more glutamic acid units. Biologically active Folates are derived from Folic acid and serves as 1-carbon donors and acceptors in many biosynthetic pathways. Folates must be reduced to tetrahydrofolates in a process catalyzed by the enzyme dihydrofolate reductase to form functional compounds. they are essential for DNA replication and cellular proliferation. Folates are heat labile and water soluble. Folate is widely available in green vegetables(spinach) fruits (orange juice) and animal organs (liver, kidney). Inadequate folate intake is the most common etiology of folate deficiency causing megaloblastic anemia.

Anemia and micronutrient deficiencies among children continue to be major public health challenges in most developing countries. Data is insufficient on the status of vitamin B 12 and folate deficiency among children in the age group 6 months to 5 years in India. There were also only few studies available in the international scenario.

MATERIALS AND METHODS

- **Study design:** The research design of the study was a hospital based observational cross-sectional study.
- **Study period**: June 2015 to till sample size completed
- **Study setting**: The study was carried out at the Department of Pediatrics Deen Dayal Upadhyay Hospital.
- **Study population**: The study subjects included all patients with Hb<10gm/dl of age group 6months to 5 years admitted in Paediatric, Deen Dayal Hospital.
- **Inclusion criterion:** All patients admitted in Paediatrics, Deen Dayal Upadhyay Hospital Hari Nagar New Delhi having their Hb < 10gm/dl in age group 6 month to 5 years.
- **Exclusion criterion:** All patients admitted as a severe anaemia with acute blood loss and all patients previously treated with vitamin B12.

Study Procedure

- All children (age 6 month- 5 year) admitted in Deen Dayal Upadhayay hospital those found to have anaemia (Hb< 10 gm/dl) was included in the study after a written informed consent from the parents.

From those enrolled in study the data was collected as follows

1. Data collection from history: Consenting parents was questioned regarding their socio-demographic profile, educational status of mothers (of children), family type (nuclear or joint family), standard of living (SLI), religion and caste, pattern of dietary food.
2. Clinical examination: A complete general examination will be done of each child and the following points were noted: -
   - Vital Parameters - Heart rate, Respiratory rate, Temperature
   - Abnormalities like pallor, glossitis, hyperpigmentation of skin, icterus, hypotonia.
   - Detailed head to toe examination was done.
   - Systemic examination was done.

3.Investigations

5 ml blood sample was collected aseptically from a superficial vein which was used for all parameters to be tested.

- ✓ CBC (complete blood count) with peripheral smear examination.
- ✓ Serum sample was collected and stored in a refrigerator at -30 to -70 degree Celsius.

All test results were recorded and used for the purpose of this study. The results were also be communicated to the guardian or parent and the doctor serving the ward.

RESULTS

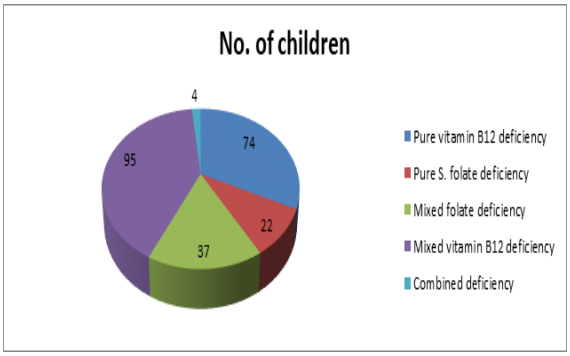

Figure 1: Distribution of Micronutrient Deficiency Among Children 6-60 Months

Table 1: Distribution of children according to age and gender

Age (In Months)	Female	Male	Total
12-Jun	34(33.7%)	67(66.3%)	101(40.07%)
13-36	59(48.7%)	62(51.3%)	121(48.01%)
37- 60	19(63.4%)	11(36.6%)	30(11.90%)
Total	112(44.45%)	140(55.56%)	252

Table 2: Distribution of children according to age and socioeconomic status

AGE (IN MONTH)	LOWER	UPPER LOWER	TOTAL
6 -12	88(87.12%)	13(12.9%)	101
13-36	82(67.7%)	39(32.3%)	121
37- 60	14(46.6%)	16(53.4%)	30
Total	184(73.01%)	68(26.98%)	252

Table 3: Distribution of Children According to Age Group and Vitamin B_{12} Level

Age group(in months)	Total	S. Vitamin B_{12} level		P VALUE
		Normal	Deficient	
12-Jun	101	57(56.43%)	44(43.56%)	
13-36	121	78(64.46%)	43(35.53%)	
37-60	30	22(73.33%)	8(8.41%)	
Total	252	157	95	0.194

Table 4: Distribution According to Age Group and S. Folate Level

Age group (in months)	total	S. Folate level		P value
		normal	deficient	
12-Jun	101	85 (84.15%)	16(15.84%)	
13-36	121	106(81.60%)	15(12.4%)	
37-60	30	24(80%)	6(20%)	
Total	252	172(68.25%)	37(14.7%)	0.524

Table 5: Distribution according to MCV and vitamin B_{12} level

MCV	Total	S. Vitamin B_{12}		P value
		Normal	Deficient	
Microcytic	208	152(73.07%)	56(27%)	0.0001
Macrocytic	35	0(0%)	35(100%)	
Normocytic	9	5(55.6%)	4(44.4%)	
Total	252	157	95	

Table 6: Distribution of children according to MCV and S. Folate level

MCV	Total	S. Folate level		P value
		Normal	Deficient	
Microcytic	208	175(83.2%)	33(15.86%)	0.0018
Macrocytic	35	35(100%)	0(0%)	
Normocytic	9	5(55.6%)	4(44.4%)	
Total	252	215	37	

Table 7: Distribution of children according to severity of anaemia and vitamin B_{12}

Anemia	Total	S. Vitamin B_{12}		P value
		normal	Deficient	
Moderate	114	73(64%)	41(36%)	0.605
Severe	138	84(61%)	54(39%)	
Total	252	157	95	

Table 8: Distribution of Children According to Severity of Anaemia and S. Folate Level

Anaemia	Total	S. Folate level		P value
		normal	deficient	
Moderate	114	102(89.5%)	12(10.5%)	0.0902
Severe	138	113(81.9%)	25(18.1%)	
Total	252	215	37	

Table 9: Distribution of Children According to S. Vitamin B_{12} Level and Presenting Complaint

Vitamin B12	total	URI/ARI	Gastroenteritis	Seizure	Nonspecific findings	P value
Normal	157	88(50.3%)	36(66.6%)	9(5.73%)	42(26.75%)	0.074
Deficient	95	60(63.15%)	18(18.94%)	6(6.31%)	11(11.57%)	
total	252	148	54	15	35	

Table 10: Distribution of Children According to S. Folate Level and Presenting Complaint

S. Folate level	Total	URI/ARI	Gastroenteritis	Seizure	Nonspecific complaints	P value
Normal	215	129(60%)	48(22.32%)	12(5.6%)	26(12.0%)	0.195
Deficient	37	19(51.35%)	6(16.21%)	3(8.1%)	9(24.3%)	
Total	252	148(58.73%)	54(21.42%)	15(5.95%)	35(13.88%)	

Table 11: Distribution of children according to vitamin b_{12} level and peripheral blood picture

Vitamin B_{12}	Microcytic	Macrocytic	Normocytic	Dimorphic	Total	P value
normal	150(95.5%)	0(0%)	4(2.54%)	3(1.91%)	157	0.0001
deficient	18(18.94%)	33(34.73%)	0(0%)	44(46.31%)	95	
total	168(66.67%)	33(13.10%)	4(1.59%)	47(18.65%)	252	

- Present cross sectional observational study was done in Pediatric department, Deen Dayal Upadhyay Hospital, Delhi. Out of 252 children 112 (44.5%) were female and 140(55.56%) were males. Maximum number of children 121(48%) were present in age group 13-36 months. Out of total 252 children, 184 (73.01%) were from Lower socioeconomic class and 68(26.98%) were from upper lower class.
- In present study the Pure or mixed vitamin B_{12} deficiency was seen 95(37.7%). Pure or mixed folate deficiency was found in 37(14.7%) children. Vitamin B_{12} deficiency 74 (29.3%) and folate deficiency 22(8.7%) alone or combination 4 (1.5%) was seen in our study.
- Cobalamin deficiency is more common as compared to folate deficiency and there is no difference among age group and prevalence of deficiency is not a good parameter of screening of vitaminB_{12} and folate deficiency. However macrocytic MCV is in useful in evaluation of vitamin B_{12} deficiency.
- There is no significance between the severity of anemia and the prevalence of vitamin B_{12} and folate deficiency.
- Vitamin B_{12} deficiency is more common in breast fed children of age group 6 12 months as compare to other age group and folate deficiency. There is no relation between sex and prevalence of vitamin B_{12} and folate deficiency.
- The most common presenting complaint was URI/ARI followed by gastroenteritis, nonspecific complaints, and seizure in Vitamin B_{12} and folate deficiency. The most common physical finding was pallor followed by hyper pigmentation of knuckle, hepatomegaly, splenomegaly, and glossitis. In peripheral blood picture dimorphic anemia is most common followed by macrocytic and microcytic anemia. Hyper segmented neutrophil has a diagnostic value in Vitamin B_{12} and folate deficiency.

Statistical Analysis: Data will be collected using structured proforma and entered a password protected Microsoft Access Database. The hard copy data forms will be verified with the guide and stored in a lockable cabinet. Upon completion of Data entry, hard copy forms will be compared with the entered data to identify errors and corrections made appropriately. Statistical analysis for categorical data was performed using Chi -square test and Fisher's exact test whichever is applicable, p-value ≤ 0.05 will be considered statistically significant. Data was analyzed using statistical software SPSS Version 20.0.

DISCUSSION

Nutritional anemia is very far common in India and developing countries, most cases of megaloblastic anemia is caused by deficiency of folate, vitamin B12 or both. Other than megaloblastic anemia, deficiencies of vitamin B12 and folic acid in children has been incriminated to cause neurodevelopmental dysfunction, abnormal movements, and failure to thrive.

Umesh K. et al in their study concluded the prevalence of vitamin B 12 was 38.3% (95%CI: 34.1-42.8%) and folate deficiency was 63.22%(95%CI:58.5-67.7%) and combined deficiency was 13.5% among the children of age group 12-59 month. Our results deos not support their results. Ray S. in their study concluded majority of children were in age group 6month - 2year. Out of 200 cases 139(69.5%) had iron deficiency.86(43.0%) subjects had vitamin B12 deficiency and 28(14 %) had folic acid deficiency. the prevalence of only iron deficiency (37.5%), only folate deficiency (45%), Only vitamin B12 (15%) and combined folate and vitamin b12 2(1%) were noticed. Our results are like their observation.

In the present study out of 252 children, MCV was microcytic in 208 children, vitamin B12 deficient were 56 (26%); macrocytic in 35 children, vitamin B12 deficient were 35(100%); normocytic in 9 and vitamin b12 deficient in 4(44.4%). It was statistically significant P(0.0001%). Bhatia P et al concluded that MCV is unreliable parameter of screening of vitamin B12 deficiency. They took 94 cases, 26 (27.7%) had raised MCV, 50 (53.2%) had normal reference MCV and 28(24.8%) had low MCV. Our results are like their observations.

In present study out of 252 children, MCV was microcytic in 208 children, S. Folate deficient were 56 (26%); macrocytic in 35 children, S. Folate deficient were 35(100%); normocytic in 9 and Folate deficient in 4(44.4%). It was statistically significant P (0.0018%). We could not found other similar study comparing S. Folate and MCV. In present study

moderate anemia was seen in 114 children, vitamin B12 deficient were 41 (36%) and severe anemia was seen in 138, vitamin B12 deficient were 54 (39%). It was not statistically significant (P value0.65). Ray S et al concluded in their study that there is no significance (P value 0.871) between severity of anemia and Vitamin B12 deficiency in children less than five year. Our results are like their observation.

In present study moderate anemia was seen in 114 children, Folate deficient were 12 (10.52%) and severe anemia was seen in 138, Folate deficient were 25 (18.11%). It was not statistically significant (P value 0.092). Ray S. et al concluded in their study that there is no significance (P value 0.81) between severity of anemia and deficiency in children less than five year. Our study supports their observation.

In present study out of the 252 children, 6- 12 month age group children 72(28.57%) were on breast feeding and vitamin B12 deficiency was seen in 34(54.16%) children while in age group 13-60 month nonvegetarian dietary habit was seen in 23(9.12%) children and vitamin B12 deficiency was present in 3 (13%) while vegetarian dietary habit was seen in 157(66.2%) children and vitamin b12 deficiency was seen in 53(33.2%) children. Taneja S. concluded the vitamin B12 deficiency was low in breast feed children in age group 6-11month than non-breast children (P value <0.001) and vitamin B12 deficiency decreases progressively with increasing age in non-breastfed children. Our results are like their observations.

In present study out of the 252 children, 6- 12 month age group children 72(25.57%) were on breast feeding and Folate deficiency was seen in 9(12.5%) children while in age group 13-60 month nonvegetarian dietary habit was seen in 23(9.12%) children and Folate deficiency was present in 2 (8.7%) while vegetarian dietary habit was seen in 157(66.2%) children and Folate deficiency was seen in 26(13.6%) children. It was not statistically significant (P value 0.503). Taneja S. et al reported that folate concentration in the 6-11 month was higher in breast feed (20.3%) than in non-breast children (5.3%). Folate concentration increases with increasing age in non-breastfed children while folate decreases with increasing age in breastfed children (P <0.001). Our results are not similar to their observations.

In present study out of the 252 children, total female was 112(44.4), and vitamin B12 deficient was seen in 39(34.8%) and total male were 140(55.6%) and vitamin b12 deficiency was seen in 56 (40%). It was not statistically significant (P value 0.399). Umesh K et al reported total male was 239(51%) and vitamin B12deficient were 88(36.8%) and total female were 230(49%) and vitamin B12 deficient were 92(40%) and there was no significance between sex and vitamin b12 deficiency (p value 0.507). our results are like they are their observations. In present study folate deficient was seen in 14(12.5%) and total male were 140(55.6%) and folate deficiency was seen in 23 (16.4%). It was not statistically significant (P value 0.399). Umesh K et al reported total male were 214(51.4%) and folate deficient were 140(36.8%) and total female were 202(48.6%) and folate deficient were 123(60.9%) and there was no significance between sex and folate deficiency (p value 0.587). Our results are like their observation.

In our study out of 252 children, 148 (59%) children were of URI/ARI and vitamin B12 deficiency was seen in 60 (63.2%); 54 (21.4%) children were of gastroenteritis and vitamin B12 deficiency was seen in 18(19%); 15(6%) children were of seizure and vitamin b12 deficiency was seen in 6(6%); 35(14%) children were of non-specific finding including paleness of body ,swelling over lower limb and vitamin B12 deficiency was present in 11(11.6%). It was not statistically significant (P 0.0704). Khandhuri U et al reported in adults that fatigue (70%), anorexia and gastritis (60%), low grade fever (50%), cardiovascular (shortness of breath), palpitations and syncope (30%) and yellowish discoloration of eyes (20%). Paresthesia's, diarrhea, hyperpigmentation, and early greying of hair were present in <10 % of patients. Renault et al reported that two infants were having normal development till 6month of age then progressively there is neuroregression and pt present with apathy, lethargy, hypotonia, pallor, poor eye contact. Our study supports their results.

In our study out of 252 children, 148 (59%) children were of URI/ARI and folate deficiency was seen in 19(51.3%); 54 (21.4%) children were of gastroenteritis and folate deficiency was seen in 6(16.2%); 15(6%) children were of seizure and vitamin b12 deficiency was seen in3 (8.1%); 35(14%) children were of non-specific finding including paleness of body, swelling over lower limb and folate deficiency was present in 9(24.3%). It was not statistically significant (P 0.0704). In present study out of 252 children, vitamin B12 deficiency was seen in 95(37.7%%) children and glossitis was seen in 15(15.7%). It was not statistically significant (p value 0.872). Folate deficiency was present in 37(14.6%) and glossitis was seen in 6(16.21). Out of 252 children glossitis was present in 41(16.27%) children. Khanduri et al reported the glossitis (29%) in megaloblastic anemic adults.

In present study out of 252 children, among 95(37.7%) vitamin B12 deficient children pallor was seen in 95 (100%) children. It was statistically significant (P 0.005). Among 37 (14.7%) folate deficient children pallor were seen in 35(94.6%) children. It was not statistically significant (P value 0.842). Gomber S. reported in their study that all pateints had pallor on examination. Khandhuri U. reported the pallor (85%) was recorded on clinical examination. Our results are like their observations.

In present study out of 252 children among 95(37.7%) vitamin B12 deficient children hyperpigmentation of knuckle, were seen in 58(61.0%) and it is statistically significant. Among 37(94.6%) folate deficient children hyperpigmentation of knuckle, was seen in 7(19%).

It was not statistically significant. Chandra J. reported in their study clinical features peculiar to megaloblastic anemia of knuckle and terminal phalanges were seen in30 - 40 % cases. Khandhuri U. in their study reported hyperpigmentation of knuckle were present in 18% patients of megaloblastic anemia. Our results are like their observation.

In our present study out of 252 children, among 95(37.7%) children 91(95.7%) children had hepatomegaly. It was statistically significant (P value 0.007). Among 37(94.6%) folate deficient children 33(89.2%) children had hepatomegaly. It was not statistically significant (p value 0.655). Gomber S.et al reported in their study that hepatomegaly was seen in 19(66.6%) cases of megaloblastic anemia in children. Ulas T. et al in their case report also find hepatomegaly with pernicious anemia. Gayathri BN et al in their study also found that hepatomegaly was seen in 29(26.92%) patients. our results support their observations.

In our study out of 252 children among 95(37.7%) vitamin B12 deficient children splenomegaly was seen in 76(80%).it was statistically significant (P value0.0001) among 37() folate deficient children splenomegaly was seen in 19(51.3%) children. It was not statistically significant (0.152). Gomber S.et al reported in their study that splenomegaly was seen in 6(21%) cases of megaloblastic anemia in children. Gayathri BN et al in their study also found that splenomegaly was seen in 37(35.5%) patients. Our results support their observations. In our study out of 252 cases among 95 (37.7%) vitamin B12 deficient children hyper segmented neutrophil was present in 62(65.26%) children. It was statistically significant (p value0.0001). In a study by Gomber S.et al reported the presence of hyper segmentation of neutrophil in all cases. Khandhuri U. et al in their study also reported the presence of hypersegmentation of neutrophil was seen in all blood films examined and ranged from 2 to 60 % of neutrophils. Our results are like their observations.

In our study out of 252 cases among 95 vitamin B 12 deficient children peripheral blood film was microcytic in 18(18.9%), macrocytic 33(34.7%), dimorphic (46.3%) and normocytic in none (0%). Gayathri et al reported the presence of dimorphic anemia (37.5%) was most common blood picture followed by macrocytic amenia (31.5%). Khandhuri U et al reported in their study both microcytic and macrocytic red cells in blood picture. Our results are like their observations.

CONCLUSION

- The prevalence of vitamin B12 is more common in age group in 6-12 months anemic children who were on breast fed as compare to other age group. The reason may be maternal deficiency of vitamin B12. So, there is a strong need to supplement the Vitamin B12 in infancy for the normal development and healthy life of children.
- MCV is not a reliable indicator for screening vitamin B12 and folate deficiency. However Macrocytic MCV is highly suggestive of vitamin B12 deficiency. As prevalence of vitamin B12 and folate deficiency is common in anemic children of age group children 6- 5 years in our study we recommend in policy making in fortification of food to prevent nutritional anemia secondary to vitamin B12 and folate deficiency.

Declarations:
Funding: None Conflicts of interest/Competing interests: None Availability of data and material: Department of Pediatrics Deen Dayal Upadhyay **Hospital Code availability:** Not applicable Consent to participate: Consent taken Ethical Consideration: There are no ethical conflicts related to this study. **Consent for publication:** Consent taken

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