# **Original Research Article**

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# Serum folate, vitamin B12 levels and clinicohematological profile in 200 anaemic children aged 1-5 years

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### **ABSTRACT**

**Background:** This study was conducted in 200 anaemic children aged 1-5 years to check their serum folate, vitamin B12 levels and their correlation with sociodemographic parameters and clinicohaematological profile.

**Methods:** Present study was a hospital based observational cross-sectional study carried out in paediatric OPD and IPD of tertiary care institute in Amritsar. Study subjects included 200 anaemic children aged 1-5 years attending paediatric OPD or admitted in IPD of paediatrics department of SGRDIMSAR, Amritsar. Serum folate, vitamin B12 levels were measured in 200 enrolled anaemic children and their correlation with sociodemographic parameters and clinicohaematological profile was studied.

**Results:** Isolated vitamin B12 deficiency was present in 22 (11%), folate deficiency in 28 (14%) and combined deficiency was present in 10 (5%) cases. Isolated vitamin B12 deficiency was more prevalent in 1-2-year age group 10 (45.5%) cases, isolated folate deficiency in 2-3 years age group 12 (42.9%) cases whereas combined deficiency was more prevalent in 4-5 years age group 6 cases (60%). Statistically significant correlation was observed between severity of anaemia and poor socio-economic status, nutritional status, rural background. There was statistically significant association between vitamin B12 deficiency and poor socioeconomic status. Folate and combined deficiency had a positive correlation with age group. Combined vitamin B12 and folate deficiency had a statistically significant correlation with severity of anaemia.

**Conclusions:** Apart from iron deficiency anaemia, vitamin B12 and folate deficiency contributes significantly in total prevalence of anaemia. Vitamin B12 and folate supplementation is equally important in prevention as well as treatment of nutritional deficiency anaemias in paediatric population.

Keywords: Anaemia, Folate, Iron deficiency, Vitamin B12 deficiency

#### **INTRODUCTION**

Anaemia is an important clinicohaematological entity encountered in our day to day clinical practice. There is reduction in the number of red blood cells or their haemoglobin content with consequent reduction in their oxygen carrying capacity which is insufficient to meet the body's physiological needs. Infancy and early childhood is a vulnerable period for development of nutritional

anaemia having a detrimental effect on the growth and development including cognitive abilities of the child.<sup>2</sup> Globally, among the preschool children prevalence of anaemia is 47.4%.<sup>3</sup> In India, prevalence of anaemia was 70% in children aged 6-59 months.<sup>4</sup>

According to NFHS-4 (National Family Health Survey), latest data shows that prevalence of anaemia has decreased by 10% during the time period between NFHS-

3 (69.4%) and NFHS-4 (58.4%). The maximum prevalence of anaemia was seen in Jharkhand (69.9%) while the least was observed in Kerela (35.6%).<sup>5</sup>

The prevalence of anaemia in state of Punjab in age group 12-23, 24-35, 36-47, 48-59 months is 72.1%, 60.8%, 48.6% and 42.0% respectively. Approximately 50% of the population suffers from nutritional anaemia as known in countries where meat consumption is low.<sup>6</sup>

Iron deficiency anaemia by far is the commonest cause of anaemia in children but vitamin B12 and folic acid also contribute to sizeable number of anaemic cases in community.<sup>7</sup> Vitamin B12 is naturally found in animal products like fish, meat, poultry and milk products.

Milk and milk products are the important sources for vegetarians. Human milk is an adequate source for breastfeeding infants. Rice, cereals, beans, leafy vegetables are good dietary sources of folate. Folate is also synthesized by the colonic bacteria and the half-life of the vitamin is prolonged by enterohepatic circulation. 10,11

The deficiency of vitamin B12 and folate is characterized by symptoms like irritability, weakness, lethargy, hypotonia, developmental delay/regression and involuntary movements. 12

Vitamin B12 and folic acid assume great importance as they can cause pancytopenia, bleeding manifestations and may be mistaken as acute leukemia or aplastic anaemia. A lot of work has been done on iron deficiency anaemia with emphasis on iron fortification of food and salt but there is paucity of literature related to vitamin B12 and folic acid deficiency as a cause of nutritional anaemia. This study was conducted keeping this fact in mind.

# **METHODS**

Study was conducted in Paediatric department of Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab to determine the prevalence of folate and vitamin B12 deficiencies in anaemic children aged 1-5 years.

Role of sociodemographic and dietary factors in their prevalence was also studied. It was a cross sectional observational study and a sample size of 200 children was taken.

Children aged 1-5 years visiting outpatient department or admitted for any illness other than those specified below with hemoglobin less than 11 gm/dl were included in this study.

Children having acute blood loss, malignancy or any other chronic illness that directly contributes to anaemia were excluded. Detailed information about dietary intake, any worm infestations and socioeconomic status was obtained. Serum folate, vitamin B12 levels were measured along with complete blood count and peripheral smear examination. Serum vitamin B12 and folate were measured with vitrosanalyzer based on enhanced chemiluminesence technology.

The severity of anaemia was graded as mild (>10 gm/dl) but below age related cut off, moderate (7-9.9 gm/dl) and severe (<7 gm/dl).<sup>13</sup> Serum vitamin B12 level of <203 pg/ml) and folate level of <4 ng/ml) were considered as deficient.

The results were analyzed for various parameters and subjected to standard statistical analysis by SSPS 18.0 using chi-square test.

#### **RESULTS**

Out of 200 anaemic cases, maximum number of cases were having moderate anaemia (119) followed by 56 cases of severe anaemia and 25 cases of mild anaemia.

Mild and moderate anaemia was more prevalent in 2-3-year age group 10 (40%) and 40 (33.6%) respectively. Severe anaemia was more commonly observed in 1-2-year age group 18 cases (32.1%) and 4-5-year age group 19 cases (33.9%). However, the association between age group and severity of anaemia was not statistically significant.

There was overall male preponderence in study group. 132 (66%) were males and 68 (34%) were females. There was no statistically significant corelation with severity of anaemia. Majority of children were from rural background 144 (72%) as compared to 56 (28%) from urban background which had a significant corelation with severity of anaemia.

Majority of patients of mild and severe anaemia were from lower middle class, 12 (48%) and 22 (39.3%) respectively. 62 (52%) children with moderate anaemia were from upper lower class. There was statistically significant correlation between socioeconomic class and severity of anaemia.

Nutritional status was normal in majority of children, 125 (62.5%). 55 (27.5%) children had grade 1 malnutrition. Grade 2 and 3 malnutrition was present in 20 cases (10%). Grade 1 malnutrition was present in 22 (18.5%) children with moderate anaemia and 32 (57.1%) in children with severe anaemia. Grade 2 and 3 malnutrition was observed in 18 cases (32.2%) with severe anaemia. There was statistically significant association between severity of anaemia and nutritional status.

147 children (73.5%) were vegetarian and 53 (26.5%) were non-vegetarians. However there was no statistically significant corelation between dietary habits and severity of anaemia.Out of 200 anaemic cases, vitamin B12 deficiency was present in 22 cases (11%), folate

deficiency in 28 cases (14%) and combined deficiency in 10 cases (5%). Maximum number of cases of vitamin B12 deficiency, 10 (45.5%), were in 1-2-year age group

followed by 6 cases (27.3%) each in 2-3- and 3-5-year age group.

Table 1: Severity of anaemia in relation to sociodemographic factors.

		Mild (n=25) n (%)	Moderate (n=119) n (%)	Severe (n=56) n (%)	Total (n=200) n (%)	P value	
Age group (years)	1-2	6 (24.0)	27 (22.7)	18 (32.1)	51 (25.5)		
	2-3	10 (40.0)	40 (33.6)	7 (12.5)	57 (28.5)	0.084	
	3-4	4 (16.0)	25 (21.0)	12 (21.4)	41 (20.5)	0.084	
	4-5	5 (20.0)	27 (22.7)	19 (33.9)	51 (25.5)		
Gender	M/F	15/10 (60/40)	81/38 (68/32)	36/20 (64/36)	132/68 (66/34)	0.70	
Residence	U/R	5/20 (20/80)	43/76 (36/64)	8/48 (16/84)	56/144 (28/72)	< 0.05	
Socio- economic status	Upper class	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
	Upper middle	2 (8.0)	4 (3.7)	7 (12.5)	13 (6.5)		
	Lower middle	12 (48.0)	44 (37.0)	22 (39.3)	78 (39.0)	< 0.05	
	Upper lower	7 (28.0)	62 (52.0)	19 (34.0)	88 (44.0)		
	Lower	4 (16.0)	9 (7.7)	8 (14.2)	21 (10.5)		
Nutritional status	Normal	24 (96.0)	95 (79.8)	6 (10.7)	125 (62.5)		
	Grade 1 PEM	1 (4.0)	22 (18.5)	32 (57.1)	55 (27.5)	< 0.05	
	Grade 2 and 3	0 (0.0)	2 (1.7)	18 (32.2)	20 (10.0)	_	
Dietary	Veg	19 (76.0)	85 (71.4)	43 (76.8)	147 (73.5)	0.652	
habits	Non-veg	6 (24.0)	34 (28.6)	13 (23.2)	53 (26.5)	0.653	

M/F: male/female; U/R: urban/rural

Table 2: Vitamin b12, folate and combined deficiency in relation to sociodemographic factors.

		Vitamin B12 deficiency N=22 n (%)	P value	Folate deficiency N=28 n(%)	P value	Combined deficiency N=10 n (%)	P value
Age group (years)	1-2	10 (45.5)	0.121	4 (14.3)	<0.05	0 (0.0)	
	2-3	6 (27.3)		12 (42.9)		3 (30.0)	
	3-4	3 (13.6)		9 (32.1)		1 (10.0)	<0.05
	4-5	3 (13.6)		3 (10.7)		6 (60.0)	
Gender	M/F	14/8 (63.6/36.4)	0.804	17/11 (60.7/39.3)	0.524	7/3 (70/30)	0.784
Residence	U/R	7/15 (31.8/68.2)	0.672	9/19 (32.1/67.9)	0.599	2/8 (20/80)	0.563
	Upper class	0 (0.0)		0 (0.0)		0 (0.0)	0.360
Socio- economic	Upper middle	0(0.0)		0 (0.0)		2 (20.0)	
	Lower middle	7 (31.81)		9 (32.14)		3 (30.0)	
status	Upper lower	15 (68.18)		18 (64.28)		4 (40.0)	
	Lower	0 (0.0)	< 0.05	1 (3.6)	0.070	1 (10.0)	
Dietary	Veg	17 (77.27)	0.679	19 (67.9)	0.468	8 (80.0)	0.639
habits	Non-veg	5 (22.72)		9 (32.1)		2 (20.0)	
Severity of anaemia	Mild	3 (13.6)	0.843	1 (3.6)	0.143	0 (0.0)	<0.05
	Moderate	14 (63.6)		21 (75.0)		1 (10.0)	
	Severe	5 (22.7)		6 (21.4)		9 (90.0)	

M/F; Male/Female; U/R: Urban/Rural

Majority of cases were of male gender 14 (63.6%) and from rural background 15 (68.2%). 14 (63.6%) cases were having moderate anaemia.

All the 22 cases belonged to lower middle and upper lower socio-economic. 17 out of 22 children with vitamin B12 deficiency were consuming vegetarian diet.

Except for socio-economic class, there was no statistically significant corelation between age, gender, rural/urban background, dietary habits and severity of anaemia with vitamin B12 deficiency. Maximum number of cases of folate deficiency, 12 (42.9%), were in 2-3-year age group followed by 9 (32.1%) in 3-4, 4 (14.3%) in 1-2 and 3 (10.7%) cases in 4-5-year age group. Majority of cases were of male gender 17 (60.7%) and from rural background 19 (67.9%). 21 (75%) cases were having moderate anaemia.

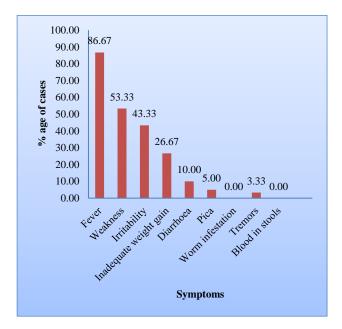


Figure 1: Clinical symptoms in vitamin B12, folate and combined deficiency (n=60).

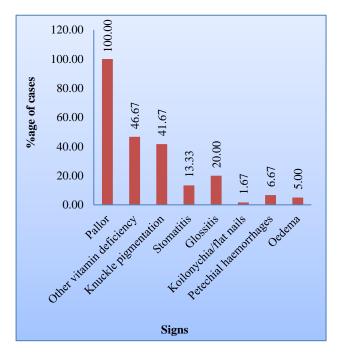


Figure 2: Clinical signs in vitamin B12, folate and combined deficiency (n=60).

All the 28 cases were from lower middle, upper lower and lower socio-economic class of society. 19 out of 28 children with folate deficiency were consuming vegetarian diet. Significant corelation of folate deficiency was found only with age group.

Out of 10 cases of combined deficiency, maximum number of cases, 6 (60%) were in 4-5-year age group followed by 4 (40%) in 2-4-year age group. Majority of cases were of male gender 7 (70%) and from rural background 8 (80%). 9 out of 10 cases (90%) were having severe anaemia. Only 1 case (10%) had moderate anaemia. Maximum number of cases 4 (40%) were from upper lower class of society followed by 3 (30%), 2 (20%) and 1 (10%) cases in lower middle, upper middle and lower class respectively. 8 out of 10 cases consumed vegetarian diet. Significant corelation of combined deficiency was found only with age group and severity of anaemia.

Table 3: Haematological parameters of patients with vitamin B12, folate and combined deficiency (n=60).

	Range	Mean± Std. Deviation
Hb (gm/dl)	2.1-10.8	7.39±2.02
PCV (%)	8.0-36.0	23.68±5.88
MCV (fl)	51.6-116.0	70.02±14.44
MCH (pg)	12.5-29.6	21.17±4.18
MCHC (gm%)	17.1-35.4	27.91±4.69
Retic Count (%)	0.01-2.00	$0.76\pm0.44$
TLC (/cumm)	3100-42100	8876.67±5243.47
Platelets (lakhs/cumm)	0.48-6.50	2.49±1.34
Vitamin B12 (pg/ml)	85-1200	270.21±181.69
Folate (ng/ml)	0.95-32.00	8.19±5.71

Out of total 60 cases of vitamin B12, folate and combined deficiency, maximum number of patients 86.7% presented with fever due to intercurrent illness followed by weakness in 53.3% and irritability in 43.3% patients. Inadequate weight gain was present in 26.67%, diarrhoea in 10%, pica in 5%, tremors in 3.3% cases. History of worm infestation and blood in stool was not detected in any of the case.

Table 4: peripheral blood smear in vitamin B12, folate and combined deficiency (n=60).

Deficiency	Microcytic hypochromic smear	Dimorphic smear
Vitamin B12 (22)	6 (27.3)	16 (72.7)
Folate (28)	24 (85.7)	4 (14.3)
Combined (10)	1 (10)	9 (90)

Pallor was observed in all the 100% cases followed by other vitamin deficiencies 46.6%, knuckle pigmentation in 41.7%, stomatitis in 13.33%, glossitis in 20%,

petechial haemorrhages in 6.67%, edema in 5% cases in flat nails/koilonychia in 1.7% cases.

Out of 60 patients with vitamin B12, folate and combined deficiency, mean haemoglobin was 7.39±2.029 gm/dl. Mean PCV was 23.68±5.88%. Mean MCV was 70.02±14.44 fl. Mean MCH was 21.17±4.18 pg. Mean MCHC was 27.91±4.69 gm %. Mean reticulocyte count was 0.76±0.44%. Mean TLC was 8876.67±5243.47/cumm. Mean platelet count was 2.49±1.34 lakhs/cumm. Mean vitamin B12 level was 270.21±181.69 pg/ml. Mean folate level was 8.19±5.71 ng/ml. Leucopenia (TLC <4000/cumm) was present in 2 cases whereas thrombocytopenia (Platelet count <1 lakhs/cumm) was present in 4 out of 60 cases.

Out of 22 cases with vitamin B12 deficiency, maximum number of cases 16 (72.7%) had dimorphic smear. Out of 28 cases of folate deficiency, majority 24 (85.7%) had microcytic hypochromic smear 24. 9 out of 10 cases of combined deficiency had dimorphic smear.

### **DISCUSSION**

Out of 200 enrolled anaemic cases in this study, maximum number of cases 119 (58.5%) were of moderate anaemia followed by 56 (28%) and 25 (12.5%) cases of severe and mild anaemia respectively. Overall male preponderance 132 (66%) was observed in this study. Majority 144 (72%) were or rural background. Malnutrition was present in 75 (37.5%) cases out of which 20 (10%) had grade 2 and 3 malnutrition. Majority of cases 88 (44%) were of upper lower class of society and 78 (39%) were from lower middle class of society.

In the present study, 60 (30%) out of 200 children had vitamin B12, folate and combined deficiency. Folate deficiency 28 (14%) was more prevalent followed by vitamin B12 deficiency 22 (11%) and combined deficiency was seen in 10 (5%) cases. Sanket K Mahajan et al observed a higher prevalence of folate deficiency (26%) as compared to vitamin B12 deficiency (6%).14 Similar observations were made by Thomas D et al who reported folate deficiency in 25% cases as compared to vitamin B12 deficiency in 4%.15 Chabbra A et al observed vitamin B12 deficiency (44%) to be more common than folate deficiency (11%).<sup>16</sup> Another study conducted by Lucia Ceuvas et al in Mexican children aged 1-6 years observed comparatively lower prevalence of folate and vitamin B12 deficiency (3.2% and 7.7% respectively).17

Out of total of 22 cases with vitamin B12 deficiency, maximum number 10 (45.5%) were in age group 1-2 years. Out of total of 28 cases with folate deficiency, maximum number 12 (42.9%), were in age group 2-3 years. Ceuvas L et al observed that highest prevalence of folate was found in 2-year-old (7.9%) and of vitamin B12 in the 1-year old (9.1%) group.<sup>17</sup> In the present study, combined deficiency was more prevalent in 4-5-year age

group (60%). Age wise distribution of isolated folate and combined deficiency was statistically significant in the present study. However, age wise distribution of isolated vitamin B12 deficiency was statistically non-significant.

Although vegetarianism was not significantly associated with folate and vitamin B12 deficiency but number of lacto-vegetarian diet consumers 44 (73.33%) were more as compared to non-vegetarian diet consumers 16 (26.66%). 77.27% children with vitamin B12 deficiency were lacto-vegetarians whereas 67.85% children with folate deficiency were lacto-vegetarians. Similar findings were observed by Khanduri U et al who observed that out of 120 patients enrolled in the study, 87% of patients with vitamin B12 deficiency and 75% patients with folate deficiency were lacto-vegetarians.18 Socioeconomic status was statistically significantly associated with vitamin B12 deficiency. Upper lower class had more prevalence of these vitamin deficiencies which was similar to findings by Kapil U et al who observed that the prevalence of folate deficiency was 22.5%, 40.4% and 52.2% respectively in the HIG, MIG, and LIG categories of adolescents while deficiency of vitamin B12 in HIG, MIG, and LIG categories of adolescent was 47.1%, 80.7% and 87.5%, respectVitamin B12 and folate deficiency was more prevalent in children with moderate anaemia (63.6% and 75% cases respectively). However, association was statistically non-significant. Combined deficiency was mostly associated with severe anaemia, 9 cases (90%), followed by moderate anaemia in 1 case (10%) which was statistically significant. Thomas D et al observed that vitamin B12 levels were deficient in 68.2% of cases with severe anaemia and 45.2% cases with mild-moderate anaemia. Similarly, folate levels were deficient in 75.6% cases with severe anaemia and 80.5% cases with mild-moderate anaemia.15 Higher proportion of severely anaemic children 56 (28%) in present studycan be attributed to hospital-based nature of the study. Difference in folate and vitamin B12 deficiency in present studyas compared to other studies done in India might be due to age groups studied.

Out of 60 children with vitamin B12, folate and combined deficiency, mean hemoglobin and PCV were 7.39±2.02 g/dl and 23.68±5.88% respectively. Mean MCV, MCH, MCHC were 70.02±14.44 fl, 21.17±4.18 pg, 27.91±4.69 gm% respectively. Mean reticulocyte count, Total leucocyte count and platelet count were  $0.76\pm0.44\%$ . 8876.67±5243.47/cumm,  $2.49\pm1.34$ lakhs/cumm respectively. Mean serum vitamin B12 and folate levels were 270.21±181.69 pg/ml and 5.71±5.72 ng/ml. Rao SS et al in their study of 33 children aged 5-15 years observed that mean hemoglobin was 7.73±1.86 gm/dl, PCV was 27.09±5.05 %, MCV was 75.90±5.63 fl, MCH was 26.30±2.12 pg, MCHC was 32.86±0.97 gm%, TLC was 11600±6400 per cumm.20 Thomas D et al observed mean hemoglobin of 9.4±2.5 gm/dl in their study. 15 Dimorphic smear was present in 16 (72.7%), 4 (14.2%) and 9 (90%) cases of vitamin B12, folate and combined deficiency respectively. Rest of the cases had microcytic hypochromic smear on peripheral blood smear examination. Gomber S et al observed that out of 29 children diagnosed with megaloblastic anaemia, peripheral smear showed predominantly macrocytes, macro-ovalocytes and pear shaped poikilocytes. Hypersegmentation of large sized neutrophils was seen in all the cases of megaloblastic anaemia. <sup>21</sup> Thomas D et al observed that out of 200 adolescents enrolled in their study, 55% had normocytic anaemia, 27.5% had microcytic, 8.5% had macrocytic and 9% had dimorphic anaemia. <sup>15</sup>

#### CONCLUSION

Present study findings are limited by the hospital-based design of the study. We conclude that apart from iron deficiency anaemia, vitamin B12 and folate deficiency contributes significantly in total prevalence of anaemia. In addition to iron supplementation, vitamin B12 and folate supplementation is equally important in prevention as well as treatment of nutritional deficiency anaemias in paediatric population.

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Institutional Ethics Committee

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