Relevance of MCV and Knuckle Pigmentation in Early Diagnosis of Vitamin B12 Deficiency in Children

Rohini Gulhane[1], Swarnika Barde[2], Swati Maldhure[3], Neelam Sukhsohale[4], Amit Gulhane[5]

[1]Assistant Professor, Department of Pediatrics, [2]Junior Resident, Department of Pediatrics, [3]Senior Resident, Department of ENT, Datta Meghe Medical College, SMHR Hospital Wanadongri, Hingna, Nagpur, [4]Associate Professor, Department of PSM, Grant Government Medical College and Sir JJ group of hospitals, Mumbai, [5]Assistant Professor, Department of Gastroenterology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi Meghe, Wardha

Abstract

Background: Vitamin B12 deficiency is common in children, especially who take strict vegetarian diet. Limited data is available on relevance of knuckle pigmentation and elevated MCV for early diagnosis in central India, especially in pediatric population.

Aim: To study association of knuckle pigmentation and elevated MCV with vitamin B 12 deficiency in children in central India

Method: This was hospital based cross sectional study, carried out from March 2019 to February 2020. 41 children with anemia (Hb < 11 gm%) or knuckle pigmentation or elevated MCV (>100 Fl) or any combination of these, were enrolled. The association of knuckle pigmentation and elevated MCV with vitamin B 12 deficiency was studied. Data was analyzed by Chi square test. P value < 0.05 was considered to be statistically significant.

Results: 29 out of 41 subjects had vitamin B 12 deficiency, maximum of which were strictly vegetarian. There was statistically significant association between vitamin b12 deficiency with elevated MCV but not with knuckle pigmentation.

Conclusion: Knuckle pigmentation and elevated MCV value may not be seen in every child with vitamin B 12 deficiency, but their presence gives important clue to early diagnosis and hence leads to early treatment

Keywords: *MCV (Mean Corpuscular Volume), knuckle pigmentation, vitamin B12 deficiency.*

Introduction

Vitamin B12 deficiency is common in India, as a majority of the population is vegetarian. Awareness about malnutrition, and anaemia is also poor among Indian mothers.[1] Vitamin B 12 deficiency is an under recognised, easily correctable cause of anemia, fatigue and neurological symptoms[2]. In children, the most common cause of megaloblastic anemia is vitamin B12 deficiency[3]. Hence childhood vitamin B12 deficiency is common in some parts of India where people follow strict vegetarian diet. Exclusively breastfed infants of vegetarian mothers are at a higher risk[4] Effective public policies are needed to support appropriate infant and young child feeding (IYCF) to ensure adequate child growth and development, especially in low and middle income countries[5]

Corresponding Author:
Dr. Rohini Gulhane
Assistant Professor, Department of Pediatrics, Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Wanadongri Nagpur–441110
e-mail: ambad.sawan@gmail.com
Mobile No.: 9890959395

A vitamin B deficiency in children (under 19 years) is defined as a serum B12 value of < 229 pmol/L or a MMA value > 0.26 to 0.29 µmol/L[6]. Dermatological manifestations of B12 deficiency include cutaneous and mucosal hyperpigmentation[7] About 19.0 % patients with megaloblastic anemia show knuckle hyperpigmentation and sometimes may be the only marker of vitamin-B12 deficiency[8] Various mechanisms have been suggested to explain hyperpigmentation associated with vitamin B12 deficiency like decreased glutathione levels activating tyrosinase and increased melanogenesis**,** defect in the melanin transfer between melanocytes and keratinocytes, resulting in pigmentary incontinence[9,10] Appropriate treatment results in dramatic clinical and laboratory response in most patients [11]

Several reports state that the incidence of macrocytosis varies from 1.7 to 3.6%. The etiology and demographic profile varies among various western and Indian studies.[12,13,14,15] Higher prevalence of macrocytosis suggests that it is one of the unrecognized and undiagnosed in routine clinical practice[16] Macrocytosis is not accompanied by anemia in upto 60% of cases; however, isolated macrocytosis should always be investigated. Macrocytosis without anemia may indicate early folate or cobalamine deficiency.[17] Macrocytosis is reported in terms of mean corpuscular volume (MCV). Normal MCV values range from 80 to 100 femtoliters (fl) and vary by age and reference laboratory.[18] In the era of automated blood cell counters, macrocytosis is a relatively common finding, with prevalence of 1.7% to 3.6%.[19,20,21] However, the sensitivity and specificity of MCV as a test for detection or exclusion of vitamin B12 deficiency is not well known

The peripheral blood smear is considered to be more sensitive than RBC indices to identify early macrocytic changes, however, MCV may underestimate macrocytosis in over 30% of cases compared to the peripheral blood smear. [22] In patients with folate or vitamin B12 deficiency, macrocytosis is the earliest abnormality seen in complete blood counts. In those patients with elevated MCV values, laboratory tests for vitamin B12 and folate deficiencies are routinely ordered . The lower limits of normal for vitamin B12 levels are not well defined, especially in pediatric age group.[23] There is paucity of data on early recognition of vitamin B12 deficiency in pediatric population in central India. Limited information is available regarding use of MCV and knuckle pigmentation for diagnosis of vitamin B12 deficiency in suburban area, in resource limited setting, hence this study is planned.

Method

The present hospital based cross sectional study was conducted in pediatric department from March 2019 to February 2020, at Shalinitai Meghe Hospital & Research Centre in collaboration with Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi Meghe, Wardha, Maharashtra. Ethical clearance was sought for from institutional ethical committee before start of the study. Children of either sex, aged between 6 months to 18 years, presented with Hb < 11 gm % or knuckle pigmentation or MCV > 100 fL or any combination of these, were enrolled in the study after taking informed consent of parents. Children with congenital bone marrow failure syndromes, leukemia, severe protein energy malnutrition, anemia due to surgical cause, infants (i.e babies < 6 month of age) were excluded from the study. In the present study, Anemia is defined as Hb < 11 gm% and categorized as mild (Hb : 9 – <11 gm%), moderate (Hb : 7- < 9 gm%) and severe (Hb: < 7 gm%). Vitamin B 12 deficiency was defined as serum vitamin B12 ≤ 180 pg/ml and furthet classified as Mild (≥ 100- ≤ 180 pg/ml), moderate (≥ 50 – < 100 pg/ml) and severe (< 50 pg/ml).

After enrolling subjects, detailed present history, relevant past history, diet history and family history was obtained. Those subjects were further investigated with CBC, peripheral smear, retic count and serum vitamin B12 level. The association of knuckle pigmentation, elevated MCV with serum Vitamin B12 level was studied. Population based variation in knuckle pigmentation and abnormally elevated MCV was observed. Appropriate treatment was started as per the standard protocol. The information collecte was recorded in a master chart. Data analysis was done using Epi info version 2.3 and percentage, mean, $\chi 2$ and P values were calculated. P value less than 0.05 was taken to denote significant relationship.

Results

Out of 41 subjects enrolled, maximum number of subjects (22, 53.6%) belonged to the age group of 12 years to 18 years, however mean age of presentation was 11.43 years. Proportion of males and females was 51.2% and 48.8 % respectively. Maximum 32 (78.04%) subjects were taking non vegetarian diet. Out of 41 enrolled subjects, 31 (75.60) subjects had anemia, of which 13 (31.7 %) subjects had mild anemia, 15 (36.5 %) had moderate anemia and 3 (7.3 %) had severe anemia. Anemia was detected accidentally in 26 (83.87%) subjects.

Vitamin B12 deficiency was observed in 29 (70.73%) subjects. 14 (34.1%) subjects had mild deficiency, 12 (29.2%) subjects had moderate deficiency and 3 (7.3%) subjects had severe deficiency. Mean value of vitamin B12 was 159 pg/ml. Severe vitamin deficiency was noted predominantly in 12 to 18 years of age group. Out of total 31 anemic subjects, 25 (80.64%) subjects were deficient in vitamin B 12. The commonest presentation of vitamin B 12 deficiency was fever (15, 51.72%) followed by generalized weakness (10, 34.4%) and paleness of body(8, 27.58%). Only one child was presented with decreased scholastic performance.

Knuckle pigmentation was present in 18 (43.90%) subjects, out of which maximum subjects 6 (33.33%) had mild vitamin B12 deficiency. Only 14 (45.16%) anemic subjects had knuckle pigmentation. The association between vitamin B12 deficiency and presence of knuckle pigmentation was not found to be statistically significant by applying chi square test with P value of 0.426.

Table 1: Table showing association of knuckle pigmentation and vitamin B12 deficiency

Knuckle pigmentation	Vitamin B 12 deficiency		P value	X^2 value
	Present	Absent		
Present (n = 18)	13	5	0.426	0.034
Absent (n = 23)	16	7		
Total (n= 41)	29	12		

Elevated MCV (> 100 Fl) was documented in only 6 (20.68%) subjects with vitamin B12 deficiency. Mean MCV value was found to be 82.26.Fl. The association between vitamin B12 deficiency and elevated MCV was found to be statistically significant with P value of 0.0015.

Table 2: Table showing association of MCV and vitamin B12 deficiency

Vitamin B 12 Deficiency	MCV		P value	X^2 value
	< 100 Fl	≥ 100 Fl		
Mild (n= 14)	12	02	0.0015	12.97
Moderate (n= 12)	11	01		
Severe (n= 3)	00	03		

On peripheral smear, macrocytes were found in only 5(17.24%) subjects. The commonest Peripheral smear finding was hypochromic microcytic with anisocytosis.

Discussion

Megaloblastic anemia is not uncommon in the Indian subcontinent as well as other parts of Asia with females and vegetarians being more susceptible to B12 deficiency. Various studies in the past have shown that occult B12 deficiency may be rather prevalent among Indian urban and rural population.[24] The diagnosis should not be dependent on abnormal blood values. Macrocytosis for example is not specific to a vitamin B deficiency in children and also anaemia is not always present – as was previously assumed. In our study, 4(9.75%) subjects were deficient in vitamin B12, but not anemic. It is estimated that if the values for haemoglobin, haematocrit and MCV are normal, more than 30% of vitamin B12 deficient patients will be missed. [25] In a Mexican study done on varied etiology of megaloblastic anemia with pre-school children, no cases had folate deficiency, while 41% had low B12 levels.[26] In a study on 45 patients with megaloblastic anemia by Bay A et al found that, 93% cases had vitamin B 12 deficiency and only 7% had folate deficiency.[27] In our study we found 70.73% subjects were vitamin B12 deficient.

Anne-Lise Bjorke Monsen et al in 2003 studied cobalamin status and its biochemical markers methylmalonic Acid and Honmocysteine in 3766

children from the age of 4 days to 19 years. They found that lowest value of serum vitamin B12 was in children with age group of 12-19 years, which is in accordance with our study in which sever vitamin B 12 deficiency (< 50 pg/ml) was predominantly seen in the age group of 12 years to 18 years. [28]

Pooled data from different studies shows that a considerable number of B12-deficient patients will remain unnoticed when the MCV is used as the only parameter to rule out the diagnosis of B 12 deficiency.[29] In our study**,** elevated MCV > 100 Fl was seen in only 6 (20.68%) subjects with vitamin B12 deficiency but the association between vitamin B12 deficiency and elevated MCV was found to be statistically significant with P value of 0.0015 and x^2 of 12.97.

A clinical study was done on 1752 children by Haken Sarbay et al.on Evaluation of children with macrocytosis found that macrocytosis in 2017. They found that macrocytosis was detected in only 14 (19.4%) in vitamin B 12 deficient children which is in accordance with our study where only 5 (17.24%) children with vitamin B12 deficiency showed macrocytosis.[30]

A retrospective analysis was conducted by S Srikant on Clinical Spectrum and a Hematological Profile of 21 children with age group from 2 months to 15 years with Megaloblastic anemia. There were no patients in the age group of 3-13 years in contrast to our study. The most common symptoms were pallor, fatigue, and neurological involvement . In another study by Katar et al.,most frequently reported clinical signs and symptoms of nutritional megaloblastic anemia were paleness, hypoactivity, attention deficit and stomatitis in young Turkish children**,** whereas the commonest presentation in our study was fever, followed by generalized weakness and paleness of body. [31,32]

Conclusions

- Knuckle pigmentation and elevated MCV value may not be seen in every child with vitamin B 12 deficiency, but their presence gives important clue to early diagnosis and hence early treatment
- Vitamin B-12 deficiency has varied manifestations and its possibility should be thought of even in absence of anemia, especially in teenage group of children.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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