e-SPEN Journal 9 (2014) e45–e48

Contents lists available at ScienceDirect

e-SPEN Journal

journal homepage: http://www.elsevier.com/locate/clnu

Short report

Vitamin-B12 and folate deficiency, major contributing factors for anemia: A population based study

Krishna Kishore Sukla [a,b,c], Rachana Nagar [a,c], Rajiva Raman [a,b,*]

[a] Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi, Uttar Pradesh, India
[b] Centre for Genetic Disorders, Banaras Hindu University, Varanasi, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 3 October 2013
Accepted 19 November 2013

Keywords:
Anemia
Iron supplementation
Vitamin-B12
Folate
Indian population
Public health

SUMMARY

Background and aims: More than 60% people in India suffer from anemia. Supplementation of iron as a state policy for decades has not brought about significant decline in its incidence. India also harbors high malnutrition rates hence it is imperative that additional nutritional components as risk factors for anemia are to be examined in Indian population. This study explores the possible association of vit-B12 and folate deficiency with the prevalence of anemia in an Indian cohort.
Methods: Hematological profile of 1290 individuals from eastern Indian states were correlated with their Homocysteine, vitamin-B12 and folate levels.
Results: Vitamin-B12 and folate deficiency were significantly associated with anemia in the studied cohort, suggesting them as vital risk factors in Indian population. Most of the macrocytic and greater than 50% of subjects with microcytic and normocytic anemia showed vit-B12 deficiency, indicating the plausible coexistence of iron and vit-B12 deficiency in the studied cohort.
Conclusions: Vitamin B12 deficiency is a compounding factor in the metabolic etiology of anemia along with folate, and this may be one of the plausible reasons for ineffectiveness of iron supplementation in reducing the incidence of anemia in Indian population. We therefore believe that there is a compelling reason to include vit-B12 & folate in the supplement regimen to alleviate the disease burden of anemia in Indian population.

1. Introduction

Anemia, a major hematological consequence of nutritional deficiency, affects maternal and fetal health, and decreased reproduction rate in adults.[1] Globally, incidence of anemia is highest in south Asia and central and west Africa.[2] Iron deficiency is the predominant cause of anemia worldwide. In India, therefore, dietary supplementation of iron is practiced and prescribed as a governmental policy since late 1970s, especially to pregnant mothers and neonates.[3] Though frequency of anemia has marginally declined in these years, the level of reduction is much less than anticipated.[3] Obviously iron deficiency alone cannot account for the high incidence of anemia in the subcontinent, and therefore other nutritional and environmental variables need to be taken into account.[4] A recent study from Pakistan shows that controlled oral supplementation of iron for more than 6 months does not result in significant decrease of anemia in school children and the authors found it plausible that other micronutrient deficiency (viz., vitamin-B12 & folate) could be a reason for ineffectiveness of iron supplementation.[5] Hence, it is imperative to check the incidence of vitamin-B12 (vit-B12) and folate deficiency anemia, especially in populations with high rate of malnutrition. An earlier study on native population from eastern India has revealed that nearly 50% of the population is deficient in vit-B12 and 11% in folate.[6] Another more recent study from the same region shows 65% of vit-B12 and 27% of folate deficiency in neonates, possibly due to poor maternal nutrition status.[7] The question we now address is how substantial is the role of vit-B12 & folate deficiency in causing anemia in this population? Analysis of the hematological profile of the same cohort shows strong association of vit-B12 (and folate) with anemia not only in individuals with macrocytosis but also those having microcytosis and normocytosis.

2. Materials & methodology

Complete blood count was measured by automated blood analyzer (Abacus Junior, Diatron, Hungary) for 1290 individuals

* Corresponding author. Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi, Uttar Pradesh, India.
E-mail address: rajiva.raman@yahoo.com (R. Raman).
[c] Equal contribution.

2212-8263/$36.00
http://dx.doi.org/10.1016/j.clnme.2013.11.003

(57.7% males; 42.3% females) from eastern Indian states. Homocysteine (Hcy), was measured by HPLC (Shimadzu, Kyoto, Japan) while vit-B12 and folate levels were measured by chemilumniscence (Immunolite1000, Siemens-Diagnostic-Products, and Flanders, NJ, USA). Biochemical data of these individuals are already published[6] and the same data have been utilized for this study which show strong correlation between elevated levels of Hcy and deficiency of vit-B12 and folate. Therefore, Hcy has been taken as a marker to indicate the micronutrient deficiency in this study. The study was approved by Institutional Ethical Committee Banaras Hindu University. Kruskal–Wallis test for comparing groups was done along with Dunns Multiple Comparison test. SPSS 16.0 version (IBM, Armonk, NY, USA) was used for statistical analysis. All the genotypes in the study were in Hardy–Weinberg equilibrium.

3. Results & discussion

The median value of hemoglobin (Hb) was 12 g/dL. Average incidence of anemia being 65%, its frequency among children, adolescents, reproductive age women and the elderly was significantly higher than in males in the age group 21–50 years (54%). Details of the age and gender-wise difference in the frequency of anemia in the studied population are given as supplementary data (Supplementary Table 1). Even though we have not tested iron levels in the studied individuals, occurrence of microcytosis along with low hemoglobin levels, both proximate indicators of iron deficiency, in 47% (391/840) of anemic subjects (Fig. 1) leaves little doubt about prevalence of iron deficiency in the studied cohort, which largely coincides with the national scenario on iron deficiency as prime cause of anemia in this cohort.

Nearly, ~50% ($n = 636$) of the subjects were deficient in vit-B12 (<220 pg/ml) and 11% (143) in folate confirming the high incidence of vit-B12 and moderate folate deficiency in the studied cohort.[6] When the samples were stratified into 4 groups: deficient (i) in Vit-B12 and folate both, (ii) only in Vit-B12, or (iii) in folate, and (iv) those with adequate levels of both the micronutrients, the frequency of individuals with low Hb and high Hcy levels in groups 1–3 was significantly higher than in group 4 even after Dunn's multiple comparison test (Table 1), confirming association of vit-B12 and folate deficiency with anemia in this cohort.

Since there is proven association of macrocytosis (MCV > 99 fL) with vit-B12 deficiency and anemia, we examined MCV of the total cohort. All the subjects having macrocytosis ($n = 142$) were anemic of whom 116 were deficient in vit-B12 and 41 in folate, some of them being deficient in both. Only 3 subjects were macrocytic and anemic despite adequate levels of these micronutrients. Out of 474 microcytics (MCV ≤ 80 fL), 391 were anemic while 190 and 31 were vit-B12 and folate deficient, respectively. In case of 674 normocytic subjects, 307 were anemic of whom 330 and 71 subjects were vit-B12 and folate deficient, respectively. 95% (181/190) of those who were vit-B12 deficient in the microcytic category were anemic just as 61% (19/31) of folate-deficient microcytics were also anemic. Among the normocytics, 72% (236/330) of vit-B12 deficient subjects were anemic while 78% (55/71) of folate deficiency individuals were anemic (Fig. 1).

While it was expected that subjects with vit-B12 and/or folate deficiency-driven anemia would have macrocytosis, that a larger proportion of them should show microcytic or normocytic anemia was surprising. We asked whether the extent of vit-B12 and folate deficiency had a bearing on the severity of anemia. Therefore the extent of vit-B12 and folate deficiencies along with HypHcy was tested in anemias with different MCVs: in microcytic anemia 46% were vit-B12 deficient, 8% folic acid deficient and 28% were HypHcy while in the case of normocytic anemia it was 77, 23 and 45% and in case of macrocytic anemia it was 82, 29 and 56%, respectively. Additionally, while a majority of macrocytic anemia subjects suffered moderate (53%) to severe (26%) anemia, most of the micro- and normocytic anemics had mild anemia (73 and 82% respectively). It indicated that vit-B12 and folate deficiency in those having macrocytic anemia could be more severe than in microcytic and normocytic anemia subjects. Also, it was a reasonable possibility that occurrence of microcytic anemia (95%) and normocytic anemia (72%) in individuals with vit-B12 deficiency could be because these individuals also suffered iron deficiency. That is, individuals with low vit-B12, if also deficient in iron, instead of macrocytic anemia would have normocytic or microcytic anemia, as also seen in other studies.[8,9]

Since, cellular availability of vit-B12 and folate is genetically regulated, we screened four known SNP's in the genes for Hcy

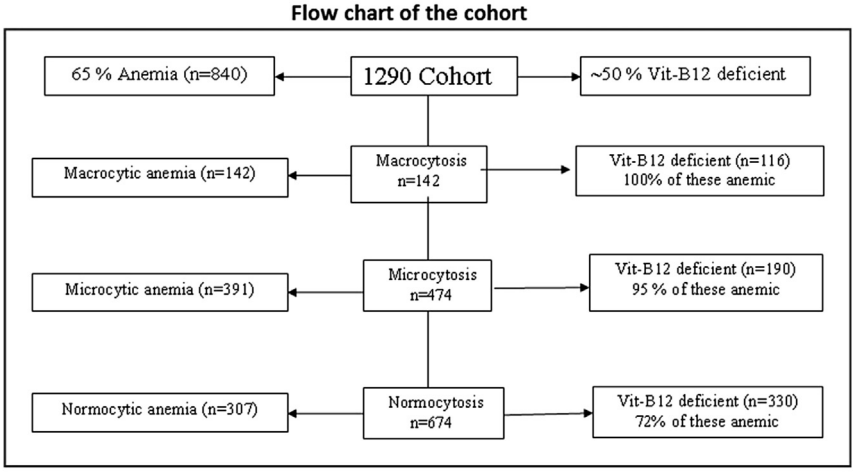

Fig. 1. Flow chart depicting the association of anemia with Vit-B12 deficiency in the studied cohort.