Original Article

To study the masking effect of folic acid on Vitamin B_{12} deficiency anemia

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Abstract

Background and Aim: A deficiency of either Vitamin B_{12} or folate can lead to megaloblastic anemia. Folate and Vitamin B_{12} metabolism is linked in transfer of a methyl group from N5-methyltetrahydrofolate to cobalamin.

Methods: This was an observational study carried out among healthy adults (students and staff) of a medical college in rural Northwest India.

Results: Despite the occurrence of suboptimal serum Vitamin B_{12} in more than half of our healthy study population on one hand, we observed normal hematological variables such as hemoglobin or lack red blood cell macrocytosis (mean corpuscular volume > 100 fL) among participants as a rule. Folic acid deficiency usually coexists with that of Vitamin B_{12}, and this has been reported by many studies in past. Strikingly, our study population had 16.98 ± 5.44 ng/ml as mean serum folic acid which was toward higher side of normal range. This sufficiency of folic acid in the group having suboptimal Vitamin B_{12} might have masked the manifestations of Vitamin B_{12} deficiency.

Conclusion: The sufficiency of folic acid in the study group might have masked the overt manifestations of Vitamin B_{12} deficiency.

Key words: Deficiency anemia, folic acid, masking, Vitamin B_{12}

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INTRODUCTION

A deficiency of either Vitamin B_{12} or folate can lead to megaloblastic anemia. Folate and Vitamin B_{12} metabolism is linked in transfer of a methyl group from N5-methyltetrahydrofolate to cobalamin. In the absence of Vitamin B_{12}, folate is "trapped" and cannot be recycled back into the folate pool. Eventually, this leads to a reduction in thymidylic acid synthesis that produces megaloblastic anemia. Serum Vitamin B_{12} has usually been seen to be lower among Indian toddlers and in adults. This is especially true in case of vegetarians. Low serum Vitamin B_{12} may not be associated with any anemia or neurological complications *per se*.[1,2] A chronic Vitamin B_{12} depletion (i.e., prolonged low intake or intestinal malabsorption) may result in a state of negative Vitamin B_{12} balance. The depletion process may take years to become clinically evident. The early and reliable diagnosis of deficiency is crucial because of a latent nature of the disorder and resulting possible irreversible neurologic damage.[3,4] Folic acid deficiency has been observed to coexist with Vitamin B_{12} deficiency.[5] Importantly, sufficiency of folic acid may have a masking effect on manifestation of Vitamin B_{12} deficiency. An extensive PubMed search revealed a paucity of reports on this in the medical literature from our part of India. Therefore, the present study was planned with the aim to assess the role of folic acid in masking Vitamin B_{12} deficiency anemia.

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MATERIALS AND METHODS

This was an observational study carried out among healthy adults (students and staff) of a medical college in rural Northwest India. The protocol and plan of the study were approved by the Institutional Ethics Committee.

Study sample

All voluntarily willing students and employees were recruited for the study after obtaining informed written consent. A total of 178 study units (students and employees) agreed to be included in the study.

Study plan and procedure were explained to the recruited participants, and they were then subjected to focused general physical education after having been interviewed using a structured questionnaire.

Blood sample was collected by following standardized procedure for the collection of blood sample, when the enrolled participant (with empty stomach after overnight fasting) came to the Department of Biochemistry next day after having got their clinical variables recorded the previous day.

Inclusion criteria

- Any voluntarily consenting student or employee aged from 18 to 62 years of the medical college.

Exclusion criteria

- Participant's refusal
- Participants who were taking methylcobalamin or Vitamin B_{12} or folic acid or iron and folic acid or have taken the same for more than 15 days during last 3 months
- Participants who had received Vitamin B_{12} or iron formulations as injectables during last 1 year
- Participants on medication for hypertension or diabetes or on lipid lowering drugs
- Those with chronic illness such as tuberculosis, cancer, or immunocompromised participants
- Pregnant or lactating mothers
- Anyone who had either donated or received blood in the last 3 months.

A total of 15 study units were excluded from the study as they fulfilled one or more exclusion criteria. Thus, a total of 153 units were included in this study.

Analysis of hematological variables such as hemoglobin, hematocrit, and mean corpuscular volume

Mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC) were analyzed within 1 h of collection of blood sample (using part of whole blood collected in the ethylenediaminetetraacetic acid pretreated tube) on KX-21 (Automated hematology analyzer, manufactured by Sysmex Corporation Kobe, Japan) by following standard operating guidelines as provided by the manufacturer. Vitamin B_{12} was estimated using a solid-phase, competitive chemiluminescent enzyme immunoassay.

Folic acid was estimated using a competitive immunoassay method of boil, competitive, liquid- phase, ligand-labeled, protein binding chemiluminescent assay with *in situ* immobilization, and with an antiligand detection system. Similarly, serum ferritin was estimated using standard procedures.

For the purpose of this study, the Vitamin B_{12} standard reference range was taken as 200–700 pg/ml, whereas the optimal range was taken as 500–1300 pg/ml.[6] Levels between 200 and 499 pg/ml were taken as suboptimal.

Statistical analysis of data

The data were entered in an excel sheet and analyzed using SPSS Statistics versions 16.0 (IBM SPSS - IBM Analytics, Armonk, North Castle, New York, United States). The data were expressed in mean (standard deviation [SD]).

RESULTS

A total of 153 study units comprising 69 employees and 84 students were enrolled in the study. Youth (18–25 years) constituted 54.91% of the study population, whereas those who were older than 45 years constituted 12.42% of the total and the rest were aged between 26 and 45 years. The oldest participant was aged 56 years. The mean ± SD age of the study participants in two groups of employees and students was: Group I, $n = 69$: 39.06 ± 8.51 years and Group II, $n = 84$: 20.82 ± 2.93 years.

None of the participants was observed to be anemic as the mean ± SD hemoglobin (Hb) concentration was 12.63 ± 1.0 g/dl and 12.20 ± 1.04 g/dl in respect of the groups under study comprising employees and students, respectively.

Strikingly, the mean serum folic acid among our participants was 16.98 ± 5.44 ng/ml, which is toward higher side of normal range [Table 1]. Another important finding in our study is that the mean Vitamin B_{12} levels are suboptimal among the study participants.

Table 1 also reveals that hemoglobin exhibited significant intergroup variance based on occupation and gender ($P < 0.05$ for each). Similar was the picture for the other variables such as hematocrit (HCT) and mean corpuscular volume (MCV) among participants. The study population had 78.89 ± 6.27 fL as mean value of MCV, and it exhibited

Table 1: Intergroup distribution of hematological and hematopoietic variables along with their deficiency markers among participants of study population (*n*=153)

Group variables	Occupation wise			Gender wise		
	Employees Group I (*n*=69)	*P*	Students Group II (*n*=84)	Male Group III (*n*=86)	*P*	Female Group IV (*n*=67)
Hb (g/dl)	12.63±1.0	0.011	12.20±1.04	12.87±0.95	0.000	11.78±0.79
Hematocrit (% age)	36.61±4.89	0.036	34.98±5.08	36.92±5.29	0.001	34.17±4.28
MCV (fl)	77.58±5.45	0.019	79.97±6.71	78.58±5.49	0.488	79.29±7.17
MCH (pg)	27.87±2.4	0.166	28.45±2.72	28.37±2.43	0.342	27.96±2.78
MCHC (g/dl)	34.64±1.8	0.010	35.34±1.40	35.07±1.87	0.668	34.96±1.3
Ferritin (ng/ml)	68.69±50.4	0.511	73.53±40.41	74.78±43.2	0.287	66.94±47.36
Vitamin B (pg/ml)	241.56±101.88	0.137	217.98±92.78	248.36±98.72	0.004	203.28±90.16
Folic acid (ng/ml)	16.77±5.27	0.67	17.15±5.6	16.66±5.33	0.417	17.38±5.6
AIFAb (EU/ml)	20.06±7.04	0.582	20.64±5.8	19.85±5.49	0.244	21.06±7.3

Hb: Hemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, AIFAB: Intrinsic factor antibodies. Data are expressed as Mean ±standard deviation. Analysis of data was done by Student unpaired '*t*' test. *P*>0.05 was considered significant

a significant occupational intergroup variation between employees and students ($P < 0.05$), but it did not show significant intergroup variation based on diet or gender. Another hematological variable, i.e., HCT having a mean value of 35.72% ± 5.05%, also showed significant intergroup variability based on occupation between employees and students ($P < 0.05$) but not based on dietary habits or gender.

Interpretation and analysis of data for one derived variable of MCH did not show any significant intergroup variability based on occupation or gender as such, but the study population had 28.19 ± 2.59 pg as its mean MCH. On the contrary, another derived parameter MCHC exhibited a significant intergroup variation ($P < 0.05$) based on occupation, i.e., between employees and students in our study [Table 1].

The study population had mean serum ferritin 71.35 ± 45.10 ng/ml. There was no significant variation of serum ferritin between different groups based either on occupation or gender.

DISCUSSION

Despite the occurrence of suboptimal serum Vitamin B_{12} in more than half of our healthy study population on one hand, we observed normal hematological variables such as Hb or lack of red blood cell (RBC) macrocytosis (MCV >100 fL) among participants. This apparently paradoxical finding raises a question as to why this disparity? The most probable reason for this, as also explained by the work done by Herbert, who labeled such type of deficiency as found in our study, to be of Stage I or II, wherein only plasma or cellular stores become depleted.[5]

Vitamin B_{12} and folic acid are essential components of DNA synthesis. Folic acid is directly involved, and Vitamin B_{12} participates as a cofactor; therefore, deficiency of Vitamin B_{12} and folic acid impairs DNA synthesis leading to reduced cell divisions and typical morphological changes in the hematopoietic precursors. The precursors are larger than cells of same stage and maturation and exhibit disparity in nuclear-cytoplasmic maturation. No participant from our study having suboptimal Vitamin B_{12} status had MCV that could be called as megalocyte; rather more than 90% of the study population had MCV within normal range (75–95 fL). Seemingly, this deficiency of Vitamin B_{12} in our study was not severe enough to cause macrocytosis or due to other situations that cause a decrement in RBC size and mask the macrocytosis.

Folic acid deficiency usually coexists with that of Vitamin B_{12}, and this has been reported by many studies in past.[4,7,8] High prevalence (25%) of folic acid deficiency was observed by Ahmed *et al*. among adolescent girls from Bangladesh.[9] Strikingly, our study population had 16.98 ± 5.44 ng/ml as mean serum folic acid which was towards higher side of normal range. This sufficiency of folic acid in the group having suboptimal Vitamin B_{12} might have masked the manifestations of Vitamin B_{12} deficiency.[10] This lack of folic acid deficiency as in our study, especially among females who had 17.38 ± 5.6 ng/ml as mean serum value was not consistent with studies conducted by other workers who reported folic acid deficiency among young adult females of childbearing age ranging from 14.3% to 26.3% of enrolled females from Iran and from an adjacent state in northern India.[11,12] We find this occurrence of isolated deficient status of Vitamin B_{12} in our study to be a unique phenomenon in itself as most of the studies at hand, report concurrent deficiency of Vitamin B_{12} along with that of folic acid.[4,13-15]

Limitations of the study

A small study sample with convenience sampling design may have limited the scope for extrapolation of the study findings to a larger population.

CONCLUSION

The sufficiency of folic acid in the study group having suboptimal levels of Vitamin B_{12} might have masked the overt manifestations of Vitamin B_{12} deficiency.

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Conflicts of interest

There are no conflicts of interest.

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