Diagnostic Accuracy of Mean Corpuscular Volume in Delineating Vitamin B₁₂ Deficiency

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Abstract

Background: Nutritional deficiency, especially B₁₂ deficiency is quite common in India. The manifestation can range from anemia usually megaloblastic anemia as an early indicator to neurological sequel that develops later. Evaluation of Mean Corpuscular Volume (MCV) as a screening parameter has been highly unreliable and misleading. This has resulted in delayed diagnosis with irreversible changes.

Aim: Our study aimed at evaluating the sensitivity, specificity and diagnostic accuracy of MCV in delineating vitamin B₁₂ deficiency.

Methods: The study population consisted of 501 adolescent girls aged 10 to 18 years going to schools in the rural areas of the district. Pre-designed proforma comprising of required demographic profile and detail history were filled-up. The cut off value used for serum B₁₂ was 200 pg/ml and that of MCV was 100 fl.

Findings: The prevalence of vitamin B₁₂ deficiency in rural areas was found to be 58.58% in adolescent school going girls. The B₁₂ deficient population depicted significant association with vegetarian diet (p<0.001) and BMI (p=0.005). The sensitivity and specificity of MCV to screen out B₁₂ deficiency were calculated to be 10.14% and 92.82% respectively. Accuracy of MCV as an indicator for B₁₂ deficiency was estimated to be 45.05%. From all the above data, the percentage of subjects missed to be diagnosed for B₁₂ deficiency of all deficient subjects was calculated to be 89.86%.

Conclusion: The study revealed that almost every alternate adolescent girl was found to be B₁₂ deficient. Raised MCV was found in only 1% cases and the sensitivity was very low. Hence, MCV should not be the screening criteria for B₁₂ deficiency which might be misleading.

Keywords: Adolescent girls; Vitamin B₁₂ deficiency; Mean corpuscular volume; Diagnostic accuracy; Sensitivity

Introduction

Many studies have evidenced for high prevalence of B₁₂ deficiency in India. Besides malnutrition, mal-absorptive disorders due to tropical sprue, gastric atrophy, Helicobacter pylori infection are among the various etiological factors for B₁₂ deficiency. Strict vegetarian diet is also a proven factor for low B₁₂ level in body [1–3]. Vitamin B₁₂ deficiency manifestation can range from anemia usually megaloblastic anemia as an early indicator to neurological sequel that develops later. For years, complete hemogram, particularly mean corpuscular volume (MCV) has been used as screening criteria for B₁₂ deficiency [4,5]. Macrocytosis, MCV>100 femtolitres (fl) often precedes anemia that occurs due to defect in red blood cell (RBC) maturation though hemoglobin synthesis proceeds normally [6,7].

Recent published data have unraveled that MCV might not be informative in subclincial B₁₂ deficiency. However, very few studies have explored regarding its diagnostic accuracy in B₁₂ deficiency. Our study aimed at evaluating the sensitivity, specificity and diagnostic accuracy of MCV in delineating vitamin B₁₂ deficiency.

Materials and Methods

The study population consisted of 501 adolescent girls aged 10-18 years going to schools in the rural areas of the district. The study was approved by the Institution Ethics Committee and requisite consent was taken from parents/legally accepted representatives (LAR). Those with history of hemolytic disease were excluded in the study. Of all 496 subjects were taken up for data analysis after excluding 5 cases with very high serum B₁₂ values who later on gave consent for being treated with B₁₂ supplementation.

Pre-designed proforma comprising of required demographic profile and detail history were filled-up. Blood sample were collected under all aseptic conditions and sent to laboratory
for analysis of complete hematogram, serum iron, and vitamin B12 levels. Complete hematogram was analyzed in automated analyzer BC-5180 from Mindray.

Serum B12 was measured in Electrochemiluminescence method in Cobas e-400 immunoassay autoanalyzer from Roche Diagnostics and serum iron was measured by Iron-Ferrozine method in Biosystem B 400 fully automated clinical autoanalyzer from Biosystem reagent and instruments.

The cut off value for serum B12 was 200 pg/ml, accordingly, the study population was categorized as B12 deficient (B12<200 pg/ml) and B12 non-deficient groups (B12 ≥ 200 pg/ml) [8]. In order to evaluate the accuracy of MCV as a screening parameter, a value of MCV>100 fl was considered as macrocytic [2].

SPSS 17 software was used for statistical analysis. Categorical data were compared using Chi-square analysis and binary-logistic regression analysis. Independent ‘t’ test was used for comparison of patients’ laboratory data of the groups. Sensitivity, specificity, positive and negative predictive value (PPV and NPV), accuracy and receiver operating curve (ROC) were calculated for MCV to verify its efficiency as screening parameter. Statistical significance was accepted at p<0.05 (Table 1).

Results

The prevalence of vitamin B12 deficiency in rural areas was found to be 58.58% in adolescent school going girls. The percentage of anemia in B12 deficient population was observed to be 43.1% (n=125/290) of which only 13 (10.4%) anemic subjects documented raised MCV. The estimated blood and serum parameters, except for serum B12 levels, neither recorded any significant differences among the two groups nor showed any significant correlation with serum B12 values. (Table 1).

| Parameters | B12 non-deficient group (n=205) | B12 deficient group (n=290) | P value |
|------------|---------------------------------|-----------------------------|---------|
| Hb         | 12.15 ± 1.24                    | 12.01 ± 1.44                | 0.25    |
| MCV        | 87.88 ± 11.72                   | 86.94 ± 10.41               | 0.35    |
| MCH        | 27.74 ± 4.53                    | 27.73 ± 7.33                | 0.97    |
| MCHC       | 30.40 ± 2.47                    | 30.46 ± 2.21                | 0.78    |
| RDW        | 14.38 ± 2.20                    | 14.76 ± 3.25                | 0.11    |
| Iron       | 70.38 ± 37.38                   | 66.77 ± 39.47               | 0.30    |

The B12 deficient population depicted significant association with vegetarian diet (p<0.001) and BMI (p=0.005) but not with other demographic profiles like age group, worm infestation and SES (Tables 2 and 3). As per logistic regression analysis, the odds of having B12 deficiency was found to be 7.67 times greater for vegetarian subjects as opposed to subjects preferring mixed diet (CI:4.59-12.82; p=0.001).

Table 2 Association of serum B12 values with diet pattern.

| Diet       | Non-deficient | B12 deficient | Total |
|------------|---------------|---------------|-------|
| Vegetarian | 19            | 135           | 154   |
| Mixed      | 186           | 155           | 341   |
| Total      | 205           | 290           | 495   |

Chi-square test p<0.001*

Table 3 Association of serum B12 values with BMI in study population.

| BMI       | Non deficient | B12 deficient | Total |
|-----------|---------------|---------------|-------|
| <18.5     | 147           | 166           | 313   |
| 18.5-25   | 56            | 117           | 173   |
| 25.1-29   | 2             | 7             | 9     |
| Total     | 205           | 290           | 495   |

Chi square test p=0.005*

The frequency of occurrence of raised MCV in the B12 deficient population was 01/100 (n=29/290). The sensitivity and specificity of MCV to screen out B12 deficiency were calculated to be 10.14% and 92.82% respectively. The PPV and NPV were respectively 65.91% and 43.01%.

Accuracy of MCV as an indicator for B12 deficiency was estimated to be 45.05%. From all the above data, the percentage of subjects missed to be diagnosed for B12 deficiency of all deficient subjects was calculated to be 89.86%. The receiver operating curve (ROC) documented an area of 0.45 indicating MCV as an unreliable test for screening (Figure 1 and Table 4).

Discussion

The prevalence of vitamin B12 deficiency in adolescent girls was found to be 58.58% which corroborated with the result published by Rajendra et al., who depicted prevalence of 50% in adolescent groups in Meeyannoor, Kerala [9]. Similarly, Kapil et al. also reported prevalence of 68.3% in children of 12 to 18 years in New Delhi [10]. In contrary, Jain et al. in their study revealed low sero-prevalence of vitamin B12 deficiency (36.5%) in Jaipur [2]. The difference in prevalence could be because the study was based on retrospective data analysis from the records, hence, the age group and also the cut-off values utilized were different. Similarly, study conducted by Kapil et al. in New Delhi, documented higher prevalence than that of us (73.5%) [11].

The reason could be attributed to diet pattern adopted by the study population. 68.95% adolescent population in our study preferred mixed diet pattern as against 46.1% in New Delhi, the reported percentage consumption of non-vegetarian diet in New Delhi as tabulated by Agrawal et al. [12].
Vegetarians were found to be 7.67 times more likely to have low B₁₂ levels than those who also prefer non-vegetarian food.

Limitations

Our study was not without any limitations. The study was based completely on serum B₁₂ levels. Serum folate was not measured because of fund constraint and also because iron folic acid supplementation are being provided to the adolescent girls which might influence the findings. Evaluation of other biochemical markers such as homocysteine and methylmalonic acid would have validated B₁₂ deficiency more accurately. However, we believe that estimation of these parameters would not be feasible in the peripheral health sector in our area because of lack of adequate instrumentation and other resources.

Conclusion

The study revealed that almost every alternate adolescent girl was found to be B₁₂ deficient. Raised MCV was found in only 1% cases and the sensitivity was very low. Hence, MCV should not be the screening criteria for B₁₂ deficiency which might be misleading.

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Conflict of Interests

The authors have no competing interests

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