Diagnostic Accuracy of Mean Corpuscular Volume in Delineating Vitamin B_{12} Deficiency

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Abstract

Background: Nutritional deficiency, especially B_{12} 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 B_{12} 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 B_{12} was 200 pg/ml and that of MCV was 100 fL.

Findings: The prevalence of vitamin B_{12} deficiency in rural areas was found to be 58.58% in adolescent school going girls. The B_{12} 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 B_{12} deficiency were calculated to be 10.14% and 92.82% respectively. Accuracy of MCV as an indicator for B_{12} deficiency was estimated to be 45.05%. From all the above data, the percentage of subjects missed to be diagnosed for B_{12} 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 B_{12} deficient. Raised MCV was found in only 1% cases and the sensitivity was very low. Hence, MCV should not be the screening criteria for B_{12} deficiency which might be misleading.

Introduction

Many studies have evidenced for high prevalence of B_{12} deficiency in India. Besides malnutrition, mal-absorptive disorders due to tropical sprue, gastric atrophy, Helicobacter pylori infection are among the various etiological factors for B_{12} deficiency. Strict vegetarian diet is also a proven factor for low B_{12} level in body [1–3]. Vitamin B_{12} 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 B_{12} 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 subclinical B_{12} deficiency. However, very few studies have explored regarding its diagnostic accuracy in B_{12} deficiency. Our study aimed at evaluating the sensitivity, specificity and diagnostic accuracy of MCV in delineating vitamin B_{12} 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 B_{12} values who later on gave consent for being treated with B_{12} 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 B_{12} levels. Complete hematogram was analyzed in automated analyzer BC-5180 from Mindray.

Serum B_{12} 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 B_{12} was 200 pg/ml, accordingly, the study population was categorized as B_{12} deficient (B_{12}<200 pg/ml) and B_{12} non-deficient groups ($B_{12} \geq 200$ 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 B_{12} deficiency in rural areas was found to be 58.58% in adolescent school going girls. The percentage of anemia in B_{12} 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 B_{12} levels, neither recorded any significant differences among the two groups nor showed any significant correlation with serum B_{12} values. **(Table 1)**.

Table 1 Comparison of mean ± SD of the estimated parameters between the two groups (Vitamin B_{12} deficient and non-deficient).

Parameters	B_{12} non-deficient group (n=205)	B_{12} 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 B_{12} 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 B_{12} 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 B_{12} values with diet pattern.

Diet	Non-deficient	B_{12} 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 B_{12} values with BMI in study population.

BMI	Non deficient	B_{12} 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 B_{12} deficient population was 01/100 (n=29/290). The sensitivity and specificity of MCV to screen out B_{12} 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 B_{12} deficiency was estimated to be 45.05%. From all the above data, the percentage of subjects missed to be diagnosed for B_{12} 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 B_{12} 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 B_{12} 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 B_{12} levels than those who also prefer non-vegetarian food.
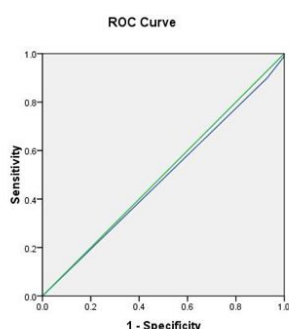

Figure 1 ROC curve for MCV to delineate deficient serum B_{12} levels.

Table 4 Area under the Curve: Tests results variable(s): indicator.

Area	Std. error	Asymptomatic sig.	Asymptomatic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.485	0.026	0.563	0.433	0.536

Due to lack of gold standard for diagnosis of B_{12} deficiency, the physicians at primary health care centers still rely on high MCV as diagnostic criterion for the same. Raised MCV was reported in only 1% of B_{12} deficient population. Khanduri et al. in their study also observed elevated MCV in only 1 subject out of 50 adults and commented that MCV was not informative [13]. Jain et al. recorded 10.36% whereas Bhatia et al. published 25% macrocytosis in B_{12} deficient subjects [1,2]. The difference could be because in the latter two studies were based on retrieval of laboratory data of serum B_{12} assays done in their respective hospitals. Other studies also suggested that raised MCV might not necessarily accompany low serum B_{12} level, hence, physicians need to be judicious enough to rule out B_{12} deficiency in case of normal MCV values [4,5,14].

The calculated sensitivity and specificity of MCV to screen out B_{12} deficiency was calculated to be 10.14% and 92.82% respectively and the diagnostic accuracy was found to be 45%. It was further calculated that nearly 89% B_{12} deficient cases would have been missed on basis of sensitivity. No significant correlation was found between serum B_{12} levels and MCV values. Also the area under ROC indicated that MCV is highly unreliable screening test. Oosterhuis et al. in their study also worked out on the diagnostic value of elevated MCV for B_{12} deficiency and reported sensitivity of 17% to 30%. Accordingly they implicated that up to 84% of B_{12} deficient cases would be missed [14]. Similarly, Thompson et al. got the finding that 82% subjects with low B_{12} levels had MCV values below 95 fL [15].

Limitations

Our study was not without any limitations. The study was based completely on serum B_{12} 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 B_{12} 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 B_{12} deficient. Raised MCV was found in only 1% cases and the sensitivity was very low. Hence, MCV should not be the screening criteria for B_{12} deficiency which might be misleading.

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Conflict of Interests

The authors have no competing interests

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