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Original Article

Pathology Section

Discriminant Functions in the Diagnosis of Vitamin B12 Deficiency Anemia, the Value of RDW-SD: An Analytical Study

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ABSTRACT

Introduction: Mean Corpuscular Volume (MCV) has been proposed as the most sensitive hematological indicator of vitamin B12 / folate deficiencies and it tends to increase in these patients even before hemoglobin levels decrease significantly. Also, there are reasonable questions as to whether discriminant functions are sufficiently accurate to warrant not ordering serum B12/folate levels and other standard tests to detect vitamin B12/folate deficiency.

Aim: This study was done to evaluate the discriminant functions MCV (Mean Corpuscular Volume), RDW-CV (Red Cell Distribution Width-Co-efficient of variation) and RDW-SD (Red Cell Distribution Width-Standard Deviation) for their ability to detect vitamin B12 deficiency and related anemia in 100 cases. We were especially interested in determining the value of a recently introduced discriminant function, RDW-SD versus RDW-CV, since so far all the attention is being given to MCV as the earliest hematologic indicator of vitamin B12 deficiency.

Materials and Methods: This was a retrospective analytical study. Total 100 patients with serum levels of vitamin B12<200 pg/mL, by Electrochemiluminescence assay (Cobas e 601, Roche diagnostics, North America) and who were evaluated for complete blood count and peripheral smear with normal serum iron profile. The hematological parameters such as hemoglobin, MCV, RDW-CV and RDW-SD were estimated by automated analyzer Sysmex XN-1000 (Sysmex America, Inc. in Lincolnshire, Illinois). Pearsons correlation was applied for calculating the correlation of hematological parameters. The p-value of less than 0.05 was considered statistically significant.

Results: Out of 100 cases of vitamin B12 deficiency, elevated RDW-SD, RDW-CV and MCV were seen in 90%, 72% and 64% cases respectively. RDW –SD showed a strong negative correlation with serum vitamin B12 levels (p-value= 0.029) which is statistically significant (p<0.05). Whereas, RDW-CV showed a weak negative correlation with serum Vitamin B12 levels with a statistically insignificant p-value of 0.58 (p>0.05). RDW-SD, RDW-CV and MCV showed a sensitivity of 95%, 81% and 69.1% respectively for detection of anemia.

Conclusion: RDW-SD is a useful discriminant function in conditions where MCV may be normal despite vitamin B12 deficiency. Standard blood reports for clinical use should include the RDW-CV and RDW-SD, in addition to MCV in diagnosis of suspected vitamin B12 deficiency.

Keywords: Mean corpuscular volume, Predictor, Red cell distribution width, Sensitive

INTRODUCTION

Standard tests used for the diagnosis of vitamin B12/folate deficiency include: Complete Blood Count (CBC) with peripheral blood smear evaluation, serum vitamin B12 and folate levels, red cell folate level, methyl malonic acid level, homocysteine level and bone marrow study [1-3]. Despite their usefulness, these standard tests are often expensive and time consuming. Routine complete blood counts and other red cell discriminant functions can be rapidly obtained from modern hematology analyzers, are inexpensive and are useful in the early diagnosis of megaloblastic anemia in low resource settings. Megaloblastic anemia secondary to vitamin B12/folate deficiency can best be detected by elevated Mean Corpuscular Volume (MCV) in a complete blood count (CBC) [1,2,4]. The MCV tends to increase in patients with cobalamin or folate deficiency even before hemoglobin levels decrease significantly [2].

Another red cell discriminant function estimated by the modern automated cell counters to measure anisocytosis is Red Cell Distribution Width (RDW) expressed as Coefficient of Variation (RDW-CV) and standard deviation (RDW-SD). While RDW-CV reflects the ratio of 1 standard deviation (SD) to the MCV, RDW-SD is the arithmetic width of the distribution curve measured at the 20% frequency curve. The normal RDW-CV is 11.5% to 14.5%. The normal RDW-SD is 39 to 47 fL [5]. RDW and MCV are considered as very useful components in predicting various anemias [6-9]. MCV, has been proposed as the most sensitive hematological indicator of vitamin B12 and folate deficiencies, regardless of hemoglobin concentration and also in evaluation of “subclinical” vitamin B12 deficiency which consists of isolated, mild biochemical changes [1,10].

Also, there are reasonable questions as to whether discriminant functions are sufficiently accurate to warrant not ordering serum B12//folate levels and other standard tests to detect vitamin B12/folate deficiency. In this study we evaluated the discriminant functions MCV, RDW-CV and RDW-SD for their ability to detect vitamin B12/folate deficiency and related anemia in 100 cases. We were especially interested in determining the value of a recently introduced discriminant function, RDW-SD versus RDW-CV, since so far all the attention is being given to MCV as the earliest hematologic indicator of vitamin B12 deficiency.

AIMS AND OBJECTIVES

This study was taken up to determine a sensitive hematological parameter indicative of vitamin B12 deficiency and to evaluate the discriminative value of MCV versus RDW in vitamin B12 deficiency. This study also aimed at evaluating the discriminative value of RDW-SD against RDW-CV as a better indicator in vitamin B12 deficiency.

MATERIALS AND METHODS

Study Design: A retrospective analytical study was undertaken in the Department of Pathology, JSS Medical College and Hospital, Mysuru, Karnataka, India over a period of one year from January 2015- December 2015.

Subjects: A total of 267 patients of vitamin B12 deficiency cases were studied over a period of six months from January 2015 to June 2015. Out of these, 100 patients satisfying the inclusion criteria were selected.

Inclusion Criteria: Patients who were detected to have low serum levels of vitamin B12 (<200 pg/mL) by Electro chemiluminescence assay (Cobas e 601,Roche diagnostics, North America) and who were evaluated for complete blood count and peripheral smear with normal serum iron profile were included in the study.

Exclusion Criteria: Cases with increased reticulocyte count (>5%), folate deficiency, liver disease indicated by deranged liver function tests, abnormal thyroid profile tests and chronic alcoholism were excluded from the study. As these conditions also are associated with macrocytosis [2].

Sample Processing: The blood sample was collected in EDTA vacutainer and the hematological parameters such as hemoglobin, MCV, RDW-CV and RDW-SD were estimated by automated analyzer Sysmex XN-1000 (Sysmex America, Inc. in Lincolnshire, Illinois) and the results were computed. Reticulocyte count was estimated by methylene blue stained smears.

Data Collection

All the results after approval were stored in hospital database. This was accessed using the inpatient number obtained from medical record section. Laboratory reference ranges considered were: macrocytosis for MCV>100fL (normal-80-100fL) [11,12], normal RDW-CV between 11.5-14.5% and RDW-SD between 39-47fL [5]. WHO reference ranges were considered for grading anemia: Anemia was defined as a hemoglobin concentration lower than 12 g/dL in women and 13g/dL in men. Mild grade anemia between 10.0 and 11.9 g/dL in women and between 10.0 and 12.9 g/dL in men; Moderate and severe anemia between 8-10.9 g/dL and <8 g/dL respectively both in men and women [13].

STATISTICAL ANALYSIS

All the data was entered in Microsoft excel sheet for analysis. Categorical variables were reported as proportions. Continuous variables were analyzed with Student t test for comparison of means. Comparison of categorical variables was done using either Chi square test or Fischer exact test as appropriate. Cramer's V test based on Pearson's Chi-squared statistic was applied for measuring the association between the nominal variables. Analysis was done using Microsoft Excel 2013 and SPSS 20. A p-value of less than 0.05 was considered statistically significant. Pearsons correlation was applied for correlation of hematological parameters.

Ethics: This study was approved by institutional ethical committee.

RESULTS

The study comprised of 100 cases of vitamin B12 deficiency consisting of 61% of males and 39% females with a mean age of 42.6 and 46.5 years, with serum levels of vitamin B12 <200 pg/mL. The lowest age was 18 yrs in both males and females, whereas the highest age was 76 years and 80 years in males and females respectively. Vitamin B12 levels ranged from 30-199 pg/ml. Considering the above mentioned reference ranges, out of total 100 cases of vitamin B12 deficiency, macrocytosis as detected by MCV (>100fL) was seen in 64% cases and 36% cases had normal MCV. Anisocytosis as picked up by RDW–CV was detected in 72% of cases and by RDW-SD in 90% of cases. Anemia as detected by decreased hemoglobin levels was seen in 81% of cases, with 8%, 24% & 49% of cases in mild, moderate and severe grades of anemia respectively, whereas 19% cases had no anemia with normal hemoglobin levels. In 19 cases of subclinical vitamin B12 deficiency with normal hemoglobin, 13 cases showed elevated RDW–SD compared to 8 and 6 cases by MCV followed by RDW-CV respectively [Table/Fig-1]. In the severe anemia group, there were no cases with normal RDW-SD and hence no false negatives with 100% sensitivity.

There was progressive increase in MCV, RDW-CV and RDW-SD with severity of anemia as shown by the increase in the mean values of each of them [Table/Fig-2]. Mean RDW-CV was 17.2% which is increased but a marked increase was seen in RDW-SD with a mean of 63.7fL.

Out of 100 cases 90 showed elevated RDW-SD while only 72 cases showed elevated RDW-CV. After applying Cramer's V test, the value was 0.229 with a significant p-value of

Hemoglobin	RDW-SD (No. of cases)		RDW-CV (No. of cases)		MCV (No. of cases)		Total Cases
	>47fL	Normal	>14.5%	Normal	>100fL	Normal	
1 (Severe anemia)	49	0	47	2	38	11	49
2 (Moderate anemia)	22	2	16	8	15	9	24
3 (Mild anemia)	6	2	3	5	3	5	8
4 (Normal/no anemia)	13	6	6	13	8	11	19
Total	90(90%)	10	72(72%)	28	64(64%)	36	100
p-value	0.001		0.001		0.016		

[Table/Fig-1]: Results of discriminant functions according to hemoglobin levels.

Anemia categories		No. of cases	Mean	Std. Deviation	Fischer's value	p-value
MCV	1 (Severe)	49	106.8531	13.03	0.541	0.655
	2 (Moderate)	24	108.5583	10.93		
	3 (Mild)	8	105.1125	15.24		
	4 (No anemia)	19	103.7842	12.97		
	Total	100	106.5400	12.64		
RDW-CV	1 (Severe)	49	17.5102	4.41	1.554	0.206
	2 (Moderate)	24	18.1375	3.41		
	3 (Mild)	8	15.3250	2.24		
	4 (No anemia)	19	16.3211	3.04		
	Total	100	17.2600	3.85		
RDW-SD	1 (Severe)	49	65.9163	17.38	1.702	0.172
	2 (Moderate)	24	65.6167	13.39		
	3 (Mild)	8	56.2875	11.86		
	4 (No anemia)	19	58.8895	13.11		
	Total	100	63.7390	15.54		

[Table/Fig-2]: Mean and standard deviations of the hematologic parameters.

0.001 [Table/Fig-3]. Hence, RDW-SD is a better discriminant function than RDW-CV in B12 deficiency cases to detect the hematological abnormality. Whereas between RDW-SD and MCV, the value was 0.309 with a p-value of <0.001 which is highly significant [Table/Fig-3] and hence to infer that RDW-SD is a more useful discriminant function and a better indicator of the hematological changes compared to MCV in cases with B12 deficiency.

		RDW-SD	RDW-CV	MCV
Increased	No. of cases	90	72	64
	%	90.0%	72.0%	64.0%
Normal	No. of cases	10	28	36
	%	10.0%	28.0%	36.0%
Total		100	100	100

[Table/Fig-3]: Chi-square based Cramer's V test for RDW-SD versus RDW-CV and RDW-SD and MCV.
RDW-SD versus RDW-CV: Cramer's Phi value:0.229, p value=0.001
RDW-SD versus MCV : Cramer's Phi value:0.309 , p value=<0.001

Pearson's correlation analysis of the discriminant functions in relation to serum vitamin B12 levels: Hemoglobin levels decrease with a fall in serum B12 levels as shown by a significant positive correlation (p<0.005). RDW–SD is a better predictor in detecting B12 deficient cases with a strong negative correlation with serum Vitamin B12 levels as shown by p-value of 0.029 which was statistically significant (p<0.05). Whereas, RDW-CV showed a weak negative correlation with serum vitamin B12 levels with a statistically insignificant p-value of 0.058 (p>0.05) [Table/Fig-4]. MCV also showed a significant negative correlation with serum vitamin B12 levels.

RDW Relationship to Haemoglobin and MCV in Vitamin B12 Deficient Cases: Pearson's correlation analysis of RDW in relation to haemoglobin (Hb) was conducted. Hb had a significant negative association (p≤0.001) with RDW-CV and RDW-SD. RDW -SD is a better discriminant function in picking up B12 deficient cases with macrocytosis, having a strong positive correlation (correlation coefficient of 0.59) with MCV shown by more clustering of dots, than RDW- CV

Variable 1 versus Variable 2		Pearson's Correlation Coefficient	p-value
HB	B12	0.218	0.029
RDW-CV	B12	-0.190	0.058
RDW-SD	B12	-0.219	0.029
MCV	B12	-0.271	0.006
RDW-CV	MCV	0.260	0.009
RDW-SD	MCV	0.591	<0.001
RDW-CV	RDW-SD	0.871	<0.001

[Table/Fig-4]: Pearson correlation applied on the hematological parameters.
Pearson correlation test (p<0.05- statistically significant)
*HB-Haemoglobin, B12-Serum vitamin B12 level, MCV-Mean Corpuscular Volume, RDW-CV (Red Cell Distribution Width-Co-efficient of variation) and RDW-SD (Red Cell Distribution Width-Standard Deviation).

that showed a weak positive correlation with MCV (correlation coefficient of 0.26) [Table/Fig-5].

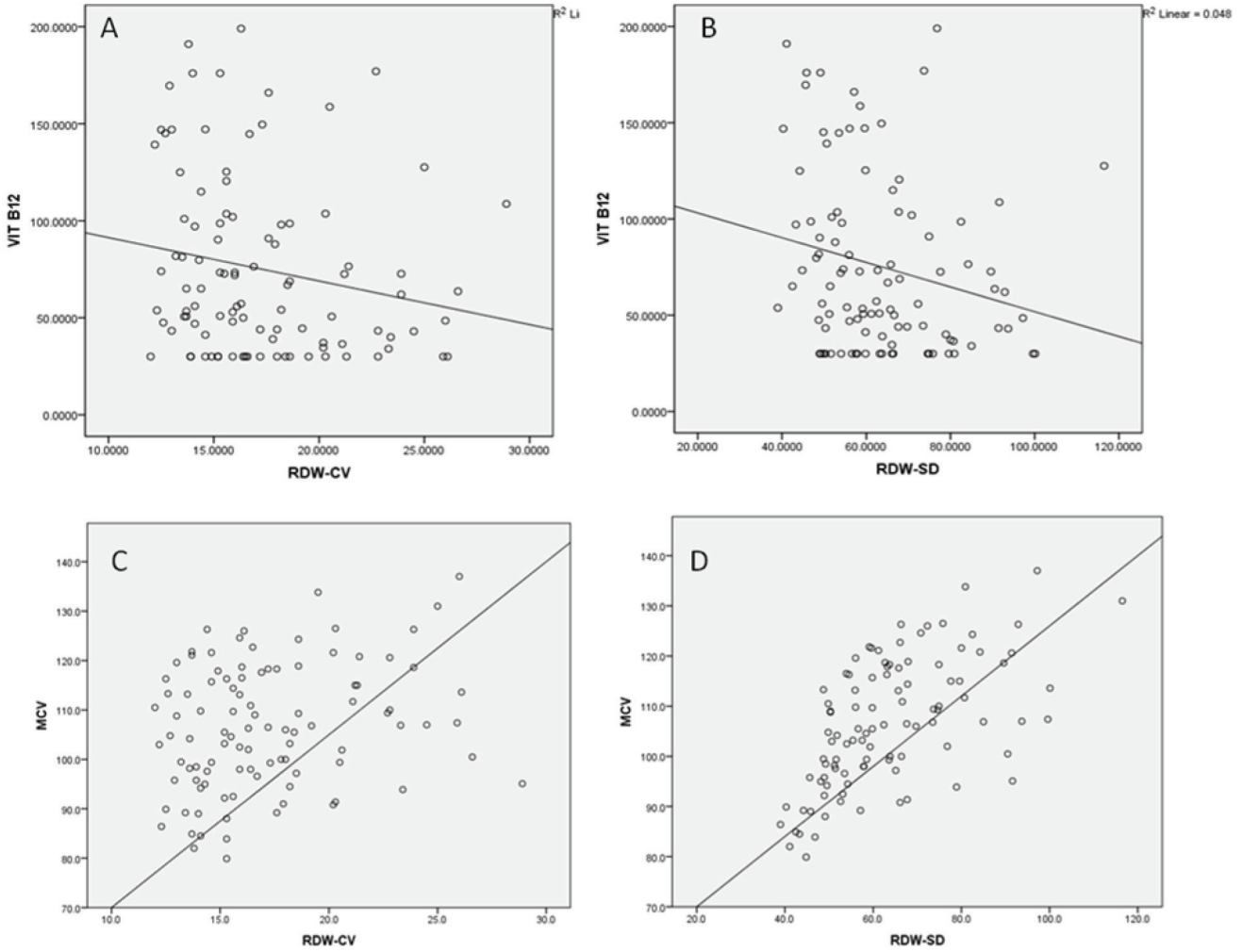
The sensitivity, specificity, positive predictive values and negative predictive values for each of the discriminant functions were calculated between vitamin B12 deficient cases with anemia and without anemia. RDW-SD showed a higher sensitivity of 95% compared to RDW-CV with 81% [Table/Fig-6].

	RDW-SD	RDW-CV	MCV
Sensitivity	95%	81%	69.1%
Specificity	31%	68%	57.8%
NPV	60%	46%	30.5%
PPV	85.5%	91%	87.5%

[Table/Fig-6]: Evaluation of RDW-SD, RDW-CV and MCV in anemia detection.

DISCUSSION

Macrocytosis reflected by Mean Corpuscular Volume (MCV) is an early diagnostic harbinger of serious vitamin B12



[Table/Fig-5]: RDW-SD and RDW-CV relationship with serum Vitamin B12 and MCV values.
[RDW-CV with serum vitamin B12:inverse r = -0.0190 p>0.005(0.058),RDW-SD with vitamin B12: inverse r = -0.0219 p <0.005(0.029), RDW-CV with MCV: linear r = 0.260 p<0.005, RDW-SD with MCV: linear r= 0.591 p<0.005]

deficiency, regardless of hemoglobin concentration [1,2]. A rising MCV precedes anemia by months and lacking clinical manifestations (including anemia and MCV elevation) [10,14,15].This subclinical deficiency accounts for almost 70% of all low serum vitamin B12 concentrations and is detected either accidentally or in population surveys [10]. The discriminant functions RDW-SD, RDW-CV and MCV were significantly higher in anemic patients increasing with the severity of anemia and to infer that they reflect the hematological changes secondary to vitamin B12 deficiency in correlation with other studies [5,11,16-18]. The study has proved that RDW -SD is a more efficient discriminant function in picking up macrocytosis than RDW-CV as showed by strong positive correlation (correlation coefficient of 0.59) with MCV, compared to RDW-CV with a weak positive correlation coefficient of 0.26, to arrive at a conclusion that erythrocyte changes of macrocytosis are best reflected by RDW-SD than RDW-CV.

Although both RDW-SD and RDW-CV use standard deviation (SD) to measure the degree of anisocytosis, cell variation is measured differently by each of them. As RDW-SD is a direct measure across the RBC histogram, it is a better and more accurate measure of anisocytosis. It is said to represent the genuine morphological/pathological status of the patients, as it encompasses the entire spectrum of MCV values [5]. Unlike in RDW-CV, those small and large abnormal cells of varying degrees of anisocytosis and/or poikilocytosis outside ± 1 SD are also included in the measurement, hence the relatively high RDW-SD results. Hence, it appears that, the higher the RDWs, the higher the degrees of anisocytosis and/or poikilocytosis [5]. This has been proved in our study that the number of cases with increased RDW-SD was more compared to RDW-CV and RDW-SD had a significant negative correlation with serum B12 levels compared to RDW-CV which was insignificant. This is a significant observation in our study that the anisocytosis in vitamin B12 deficiency were better reflected by changes in RDW-SD than RDW-CV. This finding is consistent with the study done by Caporal and Comar who evaluated RDW-SD and RDW-CV in detecting anisocytosis [16].

In the group with normal hemoglobin levels comprising of 19 cases, anisocytosis was detected in 13 cases (68.4%) by RDW-SD, while RDW-CV and MCV detected anisocytosis in 6(31.5%) and 8 cases (42.1%) respectively. Hence, we infer that vitamin B12 deficiency without anemia is better diagnosed with RDW-SD than RDW-CV/MCV. This is a significant finding in our study in contrary to other studies where MCV postulated as the most sensitive indicator of B12 deficiency [1,2,10].

This is further supported by a sensitivity of 95%, 81% and 69.1% for detecting anemia by RDW-SD, RDW-CV and MCV respectively obtained in our study. The finding that RDW–SD showed better correlation in our study as an indicator of anisocytosis even when MCV and hemoglobin levels were in the normal range, could be medically useful for detecting vitamin B12 deficiency in low resource settings. Hence, an elevated RDW-SD even in the presence of normal MCV warrants further investigations to lead to a definite diagnosis. Nevertheless, MCV and RDW-SD have shown significant negative correlation with serum vitamin B12 levels in our study [Table/Fig-5] ($p<0.005$) establishing a combined approach considering both RDW-SD and MCV in the diagnosis of anemia especially in low resource settings. Therefore, a close attention has to be given while evaluating MCV giving importance to even the RDW-SD values.

Our effort in the present study was to highlight the importance of RDW-SD as a very useful indicator in the diagnosis of macrocytic disorders, vitamin B12 deficiency being a prototype.

LIMITATION

Limitation of the study includes lack of follow-up of patients with normal hemoglobin for the future hematological changes of anemia.

CONCLUSION

High RDW-SD needs to be thoroughly investigated even in the absence of anemia and normal MCV, as it may be an indicator of underlying vitamin B12 deficiency. In a resource constraint set-up, a Complete Blood Count (CBC) is a simple, inexpensive and valuable tool in evaluating megaloblastic anemias secondary to vitamin B12 deficiency and guides the further management.

Our study is unique in that it found that RDW-SD was the most useful discriminant function compared to MCV in Vitamin B12 deficiency. Our findings were consistent with the previously reported observation that there were differences in the sensitivities of the RDW-SD and the RDW-CV and that the RDW-SD more accurately reflects the actual blood cell size variation. The fact that RDW-SD is calculated differently than the RDW-CV most likely accounts for its apparent greater value in diagnosing macrocytic anemias. Further studies are warranted in the evaluation of the RDW-SD in clinical and research setting. Most medical centers report RDW based on RDW-CV calculations. Few clinicians or public health workers know about RDW-CV and RDW-SD and the possible significance of abnormal values. Standard blood reports for clinical use should include the RDW-CV and RDW-SD, in addition to MCV in diagnosis of suspected Vitamin B12 deficiency. Hence, RDW-SD is a useful discriminant function in conditions where MCV may be normal despite Vitamin B12 deficiency.

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