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Study of BECKMAN COULTER LH 750 CPD parameters in subclinical Vitamin B12 deficiency

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Abstract

Introduction: Vit B12 deficiency causes macrocytic anemia and the early features of vitamin B12 deficiency in a peripheral blood smear is the presence of macrocytic RBCs and hyper segmented neutrophils and a raised MCV >99 fl. Subclinical deficiency is more prevalent in the population and timely diagnosis of vit B12 deficiency in such patients is of paramount importance so that timely treatment is initiated before overt clinical symptoms become evident and irreversible. The present study was undertaken to evaluate whether the Beckman coulter VCS parameters MoV –DW (monocyte anisocytosis) and NeV-DW (neutrophil anisocytosis) can predict Vitamin B12 deficiency in subclinical cases.

Materials and Methods: A total of 200 patients both male and female were included in the study. They were divided in to <20, 21-40, 41-60, 61-80 and more than 80 years age group. WHO criteria for anemia (Hb <12g/dl in women and Hb<13g/dl in men) was followed. The efficacy of hematological parameters and research data (Mean volume of neutrophils and monocytes) and their distribution width were used to detected presence of vit B12 deficiency.

Results: A total of 200 patient's data was used for this purpose. Out of 200 patients 87 (43.5%) were females and 113 (56%) were males. Male to female ratio was 1.29:1. Normal MoV-DW (8.71)NeV - DW (6.89)LyV - DW (5.86) was taken as cut off. It was observed that 50 (25%) patients had normal or near normal Hb concentration but had severe Vit B12 deficiency below 200 pg/ml. Out of these, 43 patients (86%) had MoV-DW > cut off and 40 patients had NeV-DW > cut off, 28(56%) patients had LyV-DW > cut off.

Conclusion: No single ideal marker exists for vitB12 deficiency especially in subclinical conditions. However we can say that monocyte & neutrophil anisocytosis (MoV-DW and NeV-DW) are associated with vit B12 deficiency and can be used to predict vit B12 deficiency in subclinical cases. Making use of research VCS parameters from Beckman Coulter LH750 along with complete blood count at a reasonable cost, subclinical vitB12 deficiency can be determined.

Keywords: LH 750, VCS, CPD, NeV-DW, MoV-DW, LyV-DW

Introduction

Vitamin B12 deficiency is common in vegetarian population. Vit B12 deficiency causes macrocytic anemia and the early features of vitamin B12 deficiency in a peripheral blood smear is the presence of macrocytic RBCs and hyper segmented neutrophils and a raised MCV >99 fl.[(1)]

Apart from macrocytosis, vit B12 deficiency also causes neurological symptoms which may precede the onset of overt Vit B12 deficiency.[(2)] However a raised MCV may be a spurious finding due to several other causes. Clinical manifestations of Vit B12 deficiency become evident when the levels of vit B12 fall below 100pg/ml.[(3)] However existing iron deficiency may mask these manifestations. Subclinical deficiency is more prevalent in the population and timely diagnosis of vit B12 deficiency in such patients is of paramount importance so that timely treatment is initiated before overt clinical symptoms become evident and irreversible.[(4)]

With the introduction of automated cell counters in hematology, there has been a paradigm shift in the way CBC parameters are reported. The cell population data (CPD) are measured by Beckman coulter LH 750 hematology analyzer and the study of these CPD data in different clinical conditions like viral infections, malaria, Dengue, leukemias etc. have been studied extensively. There are no special requirements and no additional cost is involved in procuring these data. These CPD are very accurate and objective than manual differential counts as nearly 8000 leukocytes are counted in their near native state by the cell counter.[(5)]

Beckman coulter LH 750 utilises VCS (Volume conductivity scatter) technology to generate these parameters. Any condition altering the morphology of leukocytes will affect the CPD volume such as myelodysplastic syndrome, chronic myeloid leukemia, Vit B12 deficiency.[(6,7,8)]

The present study was undertaken to evaluate whether the Beckman coulter VCS parameters MoV – DW (monocyte anisocytosis) and NeV-DW (neutrophil anisocytosis) can predict Vitamin B12 deficiency in subclinical cases.

Materials and Methods

A total of 200 patients both male and female were included in the study. They were divided into <20, 21-40, 41-60, 61-80 and more than 80 years age group.

WHO criteria for anemia (Hb <12g/dl in women and Hb<13g/dl in men) was followed. Blood was

collected by standard protocol in k3 EDTA for complete blood count and serum separator n tubes for vit B12 estimation. The CBC was performed on Beckman Coulter LH 750 haematology analyser and Vitamin B12 was estimated on Abbott Architect 1000 using chemiluminiscence technology. The efficacy of hematological parameters and research data (Mean volume of neutrophils and monocytes) and their distribution width were used to detecte presence of vit B12 deficiency. The WBC differential analysis on LH 750 is performed using VCS technology (Volume conductivity scatter). The mean volume of the three most abundant white cells (i.e. neutrophils, lymphocytes and monocytes) were determined (Ne MV, MoMV, LyMV). By dividing the SD of the cell volume distribution by the mean volume of each type of cells the DW was calculated.

Results

A total of 200 patient's data was used for this purpose. Out of 200 patients 87 (43.5%) were females and 113 (56%) were males. Male to female ratio was 1.29:1 Maximum patients 81 (40.5%) were in 21-40 year age group, followed by 70 (35%) in 41-60 years age group, 22 (17.5%) in 61-80 years and only one patient in more than 80 years of age. (Table 1)

Table 1: Showing demographic data of patients

Age in Year	Male	Female	Total
0-20	4	9	13
21-40	37	44	81
41-60	33	37	70
61-80	13	22	35
>80		1	1
Total	87	113	200

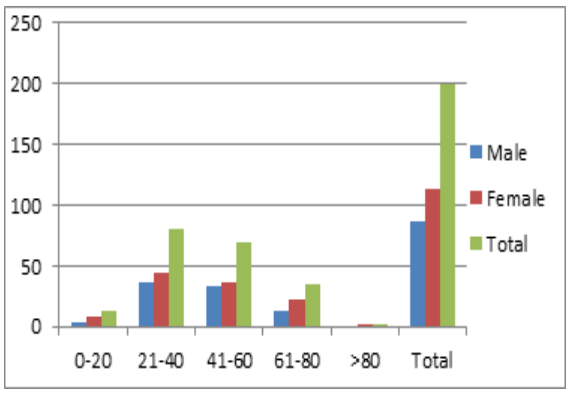

Fig. 1

Vitamin B12 data (Normal range 211-911pg/m) l was divided in to <200pg/ml, 200-500, 500-800,800-1200 and >1200 pg/ml categories (Table 2).

Table 2: Showing age wise distribution of patients in different vitamin b12 ranges

Age in Year	<200pg/ml		200-500pg/ml		500-800pg/ml		800-1200pg/ml		>1200pg/ml	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0-20	1	1	4	5	0	2	0	0	0	0
21-40	22	17	11	20	0	4	1	4	1	2
41-60	14	10	14	12	3	7	0	3	12	3
61-80	3	2	1	7	3	5	0	3	0	2
>80									1	
Total-200	40	30	30	44	6	18	1	10	14	7

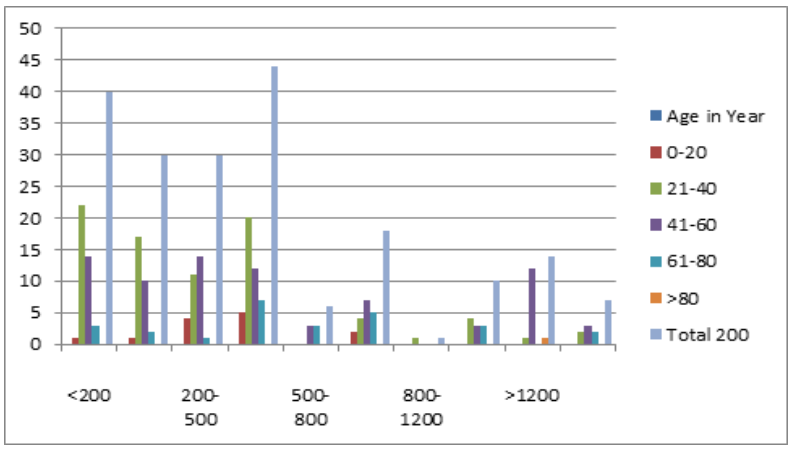

Fig. 2

74(37%) patients were in 200-500 pg/ml i.e. mild deficiency category, 70(35%) patients had severe vitamin B12 deficiency 24(12%) had Vit.B12 in range of 500-800pg /ml. 11(5.5%)had Vit B12 in 800 to 1200 pg/ml range while 21(11.5%)patients had Vit B12 more than 1200 pg/ml.

When vitamin B12 levels were compared with Hb levels (Table 3). It was observed that 40 (20%) patients had vitamin B12 less than 200pg/ml while their Hb was more than 13.0gm%. Out of these 40 patients 33 patients (82.5%) were males and 7 (17.5%) were females. 19 patients (9.5%) had Hb between 11. 1 to 13.0gm% with vitamin B12 concentration <200pg/ml. 76 patients (38%) showed mild vitamin B12 deficiency between 200-500 pg/ml rangeout of which 32 (42.1%) had Hb >13.0gm% and 44.7% had Hb between 11.0-13.0 gm%.

Table 3: Showing comparison of vitamin B12 levels with haeoglobin concentration

	<200pg/ml		200-500pg/ml		500-800pg/ml		800-1200pg/ml		>1200pg/ml	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
<5.0										
5.1-8.0	1	0	0	1	0	0	0	1	0	0
8.1-11.0	3	7	2	7	1	1	1	2	1	1
11.1-13.0	4	15	6	28	2	12	1	9	0	1
>13.0	33	7	23	9	8	6		4	2	1
Total-200	41	29	31	45	11	19	2	16	3	3

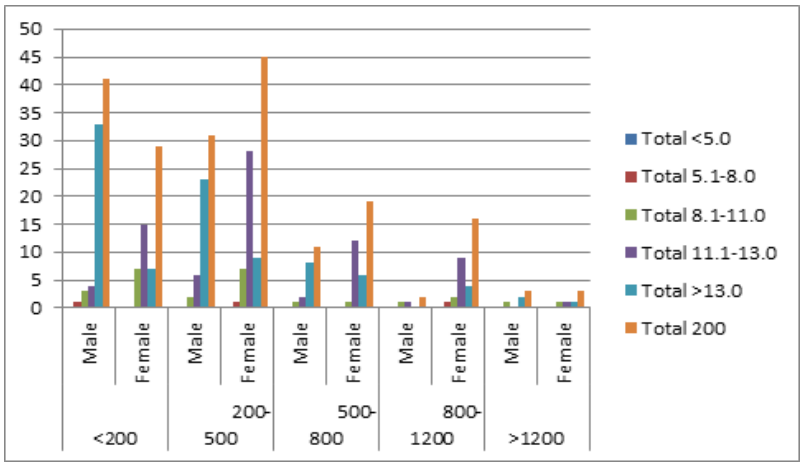

Fig. 3

30(15%) patients had vitamin B12 between500-800 pg/ml range out of which 14 had Hb >13.0gm% while 16 had Hb between 11-13 gm%. 18 patients had vitamin B12 between 800-1200pg/mland 6 patients had Vit B12 more than 1200 pg/ml. (Table 3)

1. **Comparison of Hb, Vitamin B12 and MoV-DW, NeV-DW and LyV-DW**

a. The normal MoV-DW, NeV-DW and LyV-DW was calculated from the CPD data by dividing MoV, NeV and LyV by their standard deviation(SD).

b. Normal MoV-DW(8.71)NeV - DW (6.89)LyV - DW (5.86) was taken as cut off. There were 5 patients with Hb below 9.5 gm% with severe vit B12 deficiency below 200 pg/ml. out of these 5 patients, 4 patients had MoV-DW >8.71 with average being 8.87, NeV-DW & LyV-DW did not show much variation from the normal (Table 4).

Table 4: Comparison of Low Hb & Low Vit B12 Levels

S. No	Hb<9.5gm%	Vit B12 <200pg/ml	MovV DW	NeV DW	LyVDW
1	9.3	115	9.34	5.87	5.47
2	5.5	109	7.39	6.38	2.61
3	8.1	121	9.35	7.83	4.45
4	8.4	176	7.71	6.65	6.13
5	9.4	200	10.6	6.49	6.25

c. When Hb between 9.5-11.5gm% was compared with Vit B12 levels between 200-500 pg/ml, we found that 5 patients had MoV - DW more than the cut off of 8.71 and 3 patients had NeV-DW more than the cut off of 6.89. There was no significant change in LyV-DW from the cut off (Table 5)

Table 5: Comparison of borderline HB and borderline vit B12 Levels

S. No	Hb 9.5-11.5gm%	Vit B12 200-500pg/ml	MoVDW	NeVDW	LyVDW
1	11.5	428	9.98	6.4	6.57
2	9.7	347	8.6	7.23	6.23
3	9.3	369	9.4	7.47	5.86
4	11.3	463	9.44	6.84	5.74
5	10.9	298	7.46	6.89	4.03

d. We observed that these were 16(8%) patients who had Hb > 13.00gm% and vit B12 >500 pg/ml showing MoV-DW & NeV-DW > cut off in 12 patients and 9 patients respectively. 10 patients showed LyV-DW > cut off of 5.86 in this category(Table 6).

Table 6: Comparison of normal HB with borderline vit B12 Levels

		Cutoff	Cut off 8.71	6.89	5.86
S. No	Hb> 13.0gm%	Vit B12 >500pg/ml	MovVDW	NeVDW	LyVDW
1	13.1	917	7.73	7.19	5.43
2	14.1	540	10.15	6.55	6.82
3	13.4	641	9.94	7.42	5.59
4	14.2	507	8.73	7.4	6.02
5	13.4	641	9.94	7.42	5.59
6	14.2	507	8.73	7.4	6.02
7	13.1	717	8.47	6.79	5.83
8	14.3	>2000	9.31	7.33	6.31
9	13.4	>2000	8.31	6.17	6.7
10	14.5	844	8.91	6.81	7.2
11	13.3	623	9.72	7.46	5.11
12	15.5	589	9.68	5.95	6.77
13	14.5	844	8.91	6.81	7.2
14	13.2	>2000	9.28	6.32	6.77
15	13.3	982	8.84	6.88	6.14
16	15.6	6.58	8.27	7.03	5.82

e. We compared patients with Hb >13.0 gm% with a vit B12 Concentration <200 pg/ml with their MoV-DW, NeV-DW and LyV-DW. We observed that 35 patients (17%) were in this category (Table 7).

Table 7: Showing comparison of CPD in normal Hb with severe vit.B12 Deficiency

		Cutoff	8.71	6.89	5.86
S. No	Hb >13.0gm%	Vit. <200 pg/ml	MoVDW	NeV DW	LyVDW
1	13	129	9.11	7.23	6.5
2	16.4	140	8.86	7.82	5.92
3	15.1	172	9.62	6.97	6.24
4	13.8	135	9	6.68	6.56
5	13.3	122	10.01	7.6	5.64
6	13.1	155	8.95	6.9	5.92
7	14.6	135	9.72	6.65	5.45
8	15.6	119	9.22	6.11	5.63
9	14.1	181	9.46	7.24	7
10	14.7	91	9.03	6.47	5.99
11	14.2	195	9.21	7.17	5.97
12	13.3	175	8.81	7.99	5.52
13	14.5	<83	10.12	6.69	5.49
14	15.4	94	9.33	6.95	6.38
15	13.6	0117	9.05	7.19	5.86

16	15.8	99	9.43	7.14	5.38
17	16.8	<83	10.49	7.2	6.03
18	13.6	117	9.05	7.19	5.86
19	15.8	99	9.43	7.14	5.38
20	14.9	185	9.11	6.73	6.59
21	14	179	10.85	7.98	5.59
22	13.1	108	9.03	7.04	6.38
23	13.6	<83	8.78	7.2	6.47
24	14.2	185	8.7	7.02	5.86
25	14	179	10.85	7.98	5.59
26	14.8	144	9.4	6.72	6.19
27	14	129	9.3	7.08	6.67
28	14.2	162	10.2	7.24	6.27
29	15.5	<83	9.86	6.88	4.04
30	14.1	180	6.42	7.05	5.88
31	14.4	116	10.55	6.95	6.06
32	15.8	97	9.71	7.27	5.91
33	14.4	134	8.51	7.16	6.35
34	13.9	135	9.96	6.17	5.72
35	15.3	155	8.92	7.58	4.42

f. MoV-DW was more than the cut off in 33patients NeV-DW was more than the cut off in 28 patients while 24 (68.5%) patients had LvV-DW > Cut off.

g. There were 15 patients in the category of Hb 11.0-13.0gm% with Vit B12 <200pg/ml. There were 10 patients with MoV-DW > cut off, 12 patients with NeV-DW > cut off and only 6 patients with LyV-DW > cut off (Table 8).

Table 8

		Cutoff	**8.71**	**6.89**	**5.86**
S. No	**Hb 11.0-13.0gm%**	**Vit. B12<200pg/ml**	**MoVDW**	**NeVDW**	**LyVDW**
1	12.8	133	8.13	7.03	3.84
2	12.1	154	9.9	5.71	6.29
3	11.4	111	10.2	6.94	6.33
4	12.1	152	10.18	7.56	5.26
5	12.7	195	8.39	6.99	6
6	12.8	162	8.65	7.18	6.03
7	11.3	151	9.68	8.24	5.8
8	12.7	128	8.66	6.95	5.83
9	12	<83	8.62	7.33	5.43
10	12.2	126	9.78	8.21	5.22
11	12.6	96	10.37	7.63	5.84
12	12.6	126	11.51	6.31	5.84
13	11.6	142	9.43	7.46	5.49
14	11.4	121	10.25	7.95	4.3
15	12.7	166	9.79	6.61	6.02

From the above results it was observed that 50 (25%) patients had normal or near normal Hb concentration but had severe Vit B12 deficiency below 200 pg/ml. Out of these, 43 patients (86%) had MoV-DW > cut off and 40 patients had NeV-DW > cut off, 28(56%) patients had LyV-DW > cut off.

Discussion

The most frequently used tests for determining vit B12 deficiency are vit B12 estimation and complete blood counts and their indices like macrocytosis hypersegmented neutrophils and increase in red cell distribution width (RDW).[9,10]

Vitamin B12 deficiency causes impaired DNA synthesis whereas RNA synthesis is normal. This causes macrocytosis and an increased mean corpuscular

volume. But these markers are not specific and are unreliable because the specificity of MCV is low if other causes of anemia are coexisting.[11,12]

Microscopically, hypersegmentation of neutrophils is a specific marker of vitB12 deficiency.[13]

However it has been found that vitB12 deficiency precedes the occurrence of hypersegmentation of neutrophils. So this parameters also lacks in specificity and sensitivity.

Not many studies have been conducted using these novel parameters in vitB12 deficiency. Risch C, Medine P. at al were the first to study the relationship of leukocyte anisocytosis to holotrans cobalamin deficiency.[14]

They found that MoV-DW (P=0.002) and NeV-DW (P=0.02) were significantly lower and LyMV was significantly higher (P=0.04) in patients with holo TC concentration <28 pm. In contrast, MCV, MoMV, NeMV and LyV-DW were not associated with holo TC concentrations. We observed that 50 (25%) patients had normal or near normal Hb concentration but had severe Vit B12 deficiency below 200 pg/ml. Out of these, 43 patients (86%) had MoV-DW > cut off and 40 patients had NeV-DW > cut off, 28(56%) patients had LyV-DW > cut off.

Conclusion

No single ideal marker exists for vitB12 deficiency especially in subclinical conditions. It was observed that 50 (25%) patients had normal or near normal Hb concentration but had severe Vit B12 deficiency below 200 pg/ml. Out of these, 43 patients (86%) had MoV-DW > cut off and 40 patients had NeV-DW > cut off, 28(56%) patients had LyV-DW > cut off.

There are certain limitations of our study, particularly a small sample size. A larger study group would be needed to validate our findings. However we can say that monocyte & neutrophil anisocytosis (MoV-DW and NeV-DW) are associated with vit B12 deficiency and can be used to predict vit B12 deficiency in subclinical cases. Making use of research VCS parameters from Beckman Coulter LH750 along with complete blood count at a reasonable cost, subclinical vit B12 deficiency can be determined. Additional large studies are needed to evaluate and validate the role of these research parameters for screening patients with subclinical Vitamin B12 deficiency.

Conflict of interest: None

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