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USE OF MYELOPEROXIDASE INDEX AS A SUITABLE TOOL TO MONITOR RESPONSE TO THERAPY IN PATIENTS WITH MEGALOBLASTIC ANEMIA

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ABSTRACT

Repeated bone marrow examination was found to be of value in assessing response to treatment in megaloblastic anemia. The objective of this study was monitoring the response of megaloblastics to treatment, concerning the location of neutrophilic myeloperoxidase and myeloperoxidase index (MPXI) and their variation in megaloblastic erythroid progeny. It is possible to follow up megaloblastic cases using a Technicon H1 (Bayer) automated cell counter. Complete blood counts (CBC) of 50 patients whose bone marrow aspirations revealed megaloblastic state and subsequently responded to treatment with B12 and folate were studied through pre- and post-treatment measurements. MPXI level in 41 patients was above normal range (normal: -10 to +10). Nine patients had normal MPXI, though mean cell volume (MCV) was higher than 100 fL. The highest value of MPXI was 41.5. The mean values of MPXI were 18.3 and 2.05 in pre- and post-treatment measurements respectively. MPXIs were decreased after treatment in 92% of patients (p= 0.008). According to this investigation, the MPXI measurement is a suitable test in monitoring the response for treatment.
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INTRODUCTION

Macrocytic anemias are classified as megaloblastic anemias resulting from disorders of synthesis of erythrocyte precursors in bone marrow or nonmegaloblastic anemias caused primarily by alcoholism, liver diseases and hypothyroidism.[1] In megaloblastic anemias, the mean cell volume (MCV) may exceed 150 cu fL. And the macrocytes tend to be oval.[2] Macrocytosis may be obscured or masked by coexisting iron deficiency, inflammatory diseases or thalassemia minor.[3] Occasionally, the MCV may be normal in patients with megaloblastic anemias, even in the absence of concomitant iron deficiency or thalassemia.[4] Serum vitamin B12 determination remains the best test for unmasking vitamin B12 deficiency.[2] Inspection of the blood smear for the presence of macroovalocytes and hypersegmented neutrophils remains necessary in the interpretation of MCV elevations.[4] Additional red cell information is now available using the series of automated blood cell analyzers (Ames Technicon Division of Bayer Diagnostics).[5] The MCV often returns to normal most rapidly in the most severely megaloblastic patients due to a very short mean red cell life span.[6] Repeated bone marrow examination was found to be of value in assessing response to treatment.[7]

Considering the location of neutrophilic myeloperoxidase and myeloperoxidase index (MPXI) and its variation in megaloblastic erythroid progeny, it is possible to monitor the response of megaloblastic patients to treatment. The myeloperoxidase index can be measured by Technicon H1 (Bayer) automated cell counter. Myeloperoxidase is synthesized in the promyelocyte where it is packed into azurophilic granules. During each of three subsequent

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cellular divisions, the number of azurophilic granules is halved. So the granules are divided among four progenies. The biologic basis for an elevated MPXI in megaloblastosis appears to be the presence of an increased number of myeloperoxidase-laden granules in neutrophils due to skipped cellular divisions during maturation.[8] The elevated MPXI observed in megaloblastic patients is likely to reflect the degree of cytoplasmic maturation. An elevated MPXI was reported to be a useful indicator of possible megaloblastic erythropoiesis, although normal results do not exclude a deficiency state.[9] In this project, a prospective study was performed to evaluate the range of MPXI in megaloblastic anemia and its normalization during treatment.

MATERIAL AND METHODS

Fifty patients with megaloblastic anemia referring to our hospital due to anemia or other blood disorders participated in this study. Peripheral smear and bone marrow aspirations were studied to assess morphological changes and iron stores in the bone marrow. They were shown to have typical megaloblastic features including hypersegmented polymorphonuclears and macroovalocytes in peripheral smears. Megaloblastic findings were observed in the bone marrow aspiration slides. CBC was performed using Technicon H1 system, which measures peroxidase activity and cell size through light absorbance, and light scattering as each leukocyte flows through a mercury arc light beam. The mean peroxidase activity of the neutrophil population was computed and reported as a relative deviation from the mean of a normal population, having the manufacturer's normal range for MPXI to be -10 to +10.[1] Hemoglobin (Hb), hematocrit (Hct), white blood count (WBC) and MPXI were detected before and after appropriate treatment with B12 and folate.

Vitamin B12 and folate contents of serum or red cells were not measured. Patients with no response to the standard therapy were excluded from the study. In those cases, the bone marrow biopsy and other examinations revealed that they had aplastic anemia or myelodysplastic syndrome with megaloblastoid changes in their bone marrow smear. Patients with positive response to treatment were screened regularly after appropriate treatment. Their CBCs were performed using Technicon H1 instrument along with checking MPXI and the other parameters. Paired samples were used for T-test analysis to screen MPXIs before and after treatment (p= 0. 008). MPXI was also detected in 50 normal samples using Technicon H1. The results were shown to be within normal range (-10 to +10). No changes were observed in the range of MPXI among smokers.

RESULTS

The pre- and post-treatment values of Hb, MCV, and MPXI shown in Table I. The relationships of the pre-treatment values for MPXI and MCV (MPXIl and MCV1) are shown in Figure 1, and those of post-treatment (MPXI2 and MCV2) in Figure 2. All MPXI values were shown to be above normal range in pre-treatment measurements except for 9 cases, while they had MCV values above 100 fL. In other words, although 18% of megaloblastic anemias had normal pre-treatment MPXI, but their MCV values were above normal. MPXI values were above normal range in 82% of patients. All MPXI values were decreased after treatment except in two cases.

MPXI values were decreased after treatment in 96% of patients (p= 0.008). The highest and the lowest values for MPXI were 41.5 (case 24) and -1.4 (case 42) respectively. The mean pre-MPXI and mean post-MPXI values were computed as 18.3 and 2.05 respectively. Six patients were shown to have MCV values of less than 100 fL. in pre- and post-treatment measurements along with elevated values of MPXI.

DISCUSSION

Macrocytic anemias could be divided into two groups of megaloblastic and non-megaloblastic by means of morphologic and biochemical criteria. In megaloblastic anemias, the retarded DNA synthesis results in unbalanced cell growth.

RNA synthesis remains unimpaired while cell division is restricted. As a result, cytoplasmic components, especially hemoglobin, are synthesized in excessive amounts during the delay between cell divisions. An enlarged cell is the consequence of deficiency of either vitamin B12 or folate or both.[8] Macrocytosis characteristically precedes the development of anemia and may even do so by several years. The mean cell volume (MCV) usually rises up to 110 and 130 fl., but may reach as high as 160 fl. with extreme degrees of anemia.[8] In this study six patients had MCV lesser than 100 fl. Table 1 shows significant differences for Hb and MCV values, before and after treatment, hence all Hb levels were increased and all MCV values, except in 4 patients, were decreased after treatment. In this study, MPXI was above normal range in 82% of patients. Our patients all had normal iron content except in case 34. Case 38 had megaloblastic anemia with normal MCV and elevated MPXI. His MCV decreased further after treatment and was ranged below normal. Hemoglobin electrophoresis revealed that he had thalassemia minor. Only one patient had elevated post-treatment MPXI.[25] In other words, in 98% of cases MPXI value was reduced to normal after treatment. The mean hemoglobin contents of patients in pre- and post-

Table I: Pre- and post-treatment values of the results.

	Hb1	Hb2	MCV1	MCV2	MPXI1	MPXI2
1	8.2	11.9	133.1	98.0	6.4	-2.3
2	4.9	15.0	110.4	88.2	14.5	5.4
3	7.7	9.7	118.2	117.9	24.5	2.4
4	5.8	12.1	104.2	90.7	31.6	3.2
5	7.0	13.1	105.5	81.3	12.4	5.7
6	8.0	13	112.4	93.9	22.8	- 0.6
7	5.4	11.9	123.2	90.0	22.7	1.8
8	8.8	13.7	87.0	87.7	10.2	- 2.1
9	6.5	15.9	119.1	87.2	18.9	- 5.5
10	3.7	12.5	107.4	94.5	31.9	1.4
11	8.1	12.6	114.7	102.6	9.5	- 4.0
12	6.4	11.3	124.3	105.7	19.2	3.2
13	6.9	9.8	112.6	109.1	28.8	6.8
14	6.2	17.0	103.3	87.9	29.5	0.1
15	6.7	12.7	108.1	94.4	26.4	8.3
16	5.6	13.2	124.1	87.0	12.4	5.3
17	5.4	14.9	114.4	97.9	7.7	- 5.3
18	8.9	12.1	124.9	111.0	18.7	5.4
19	4.5	12.7	109.3	94.0	20.9	7.1
20	9.1	15.6	140.7	88.3	12.9	- 4.1
21	8.1	13.3	122.3	81.7	- 1.0	- 9.0
22	5.3	13.5	119.0	92.8	17.1	- 2.6
23	7.3	13.9	112.8	83.1	10.7	8.3
24	6.2	17.7	98.9	88.6	41.5	7.6
25	3.3	12.6	82.4	91.5	28.3	45.4
26	4.8	12.8	121.1	90.8	8.0	- 3.5
27	6.9	13.2	102.3	87.4	22.2	- 5.9
28	6.2	15.0	114.1	86.9	8.2	0.6
29	3.2	12.9	125.0	89.8	32.1	7.4
30	9.9	10.8	101.1	79.2	17.4	4.4
31	7.8	11.1	121.6	84.1	17.3	1.5
32	11.3	12.4	100.1	90.7	0.4	1.9
33	8.9	15.1	122.4	75.5	12.2	- 8.5
34	6.7	11.3	94.6	90.2	25.4	4.5
35	4.6	12.1	109.1	96.3	27.2	- 2.8
36	7.0	12.1	124.9	77.1	12.4	3.5
37	5.8	10.8	114.7	88.9	23.8	0.9
38	5.8	13.8	76.7	55.4	21.1	- 1.1
39	7.9	12.5	123.9	98.6	4.5	- 8.5
40	8.3	13.3	116.6	90.9	38.8	3.9
41	5.2	11.6	102.6	84.3	24.6	2.5
42	8.5	16.1	122.O	81.1	- 1.4	- 4.7
43	8.8	13.4	80.1	81.5	10.1	3.9
44	11.4	13.5	115.1	90.0	14.4	4.4
45	8.9	12.1	127.4	99.8	12.2	6.1
46	6.6	10.2	107.0	110.2	24.1	1.1
47	8.5	12.7	134.5	108.0	17.5	4.3
48	7.3	12.9	124.4	108.5	10.1	1.1
49	5.3	16.0	107.6	95.9	31.1	- 1.3
50	5.2	13.4	123.7	94.6	23.3	4.9

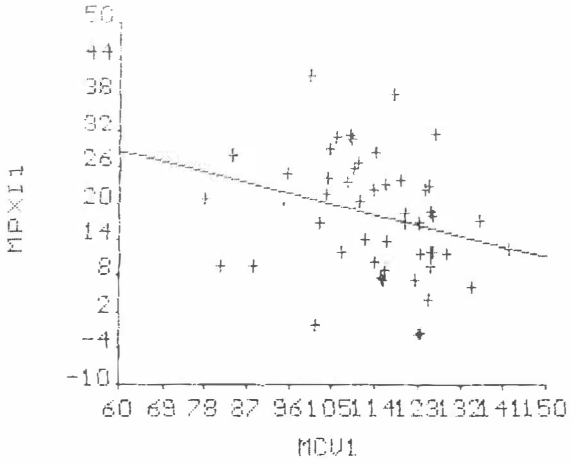

Fig. 1. The relationship of pre-treatment values for MPXI and MCV (MPXI1 and MCV1).

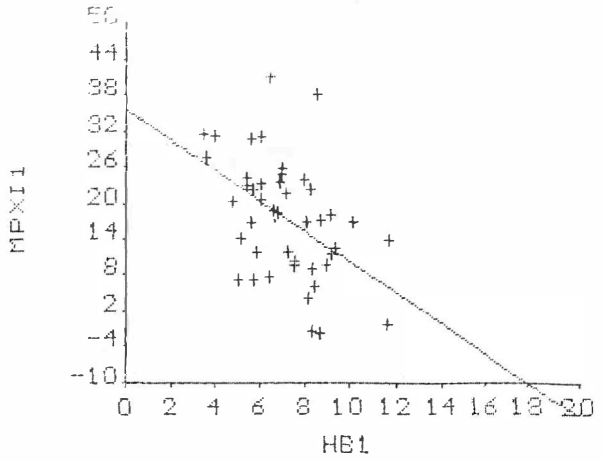

Fig. 3. The relationship of pre-treatment values for MPXI and HB (MPXI1 and Hb1).

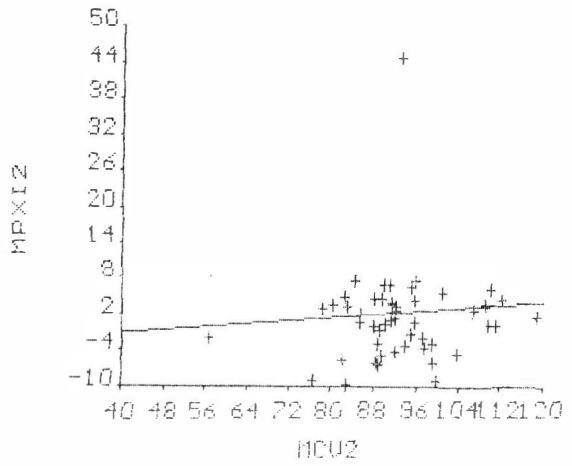

Fig. 2. The relationship of post-treatment values for MPXI and MCV (MPXI2 and MCV2).

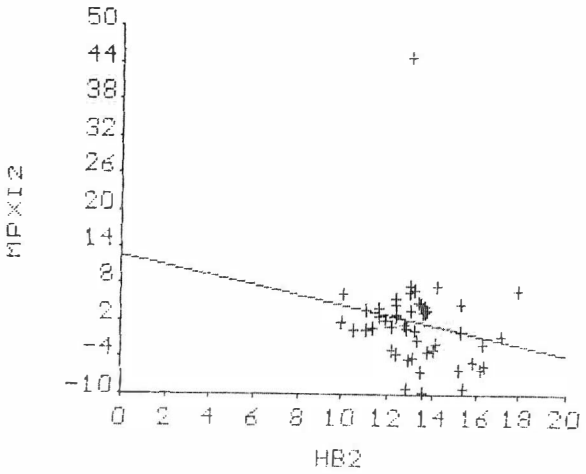

Fig. 4. The relationship of post-treatment values for MPXI and HB (MPXI2 and Hb2).

treatment measurements were shown to be 8 and 13 gr/dL respectively. Hb and MCV levels, which were abnormal in the majority of patients, were normalized after treatment. This was also experienced in 80% of 50 patients having high MPXI levels who recovered after therapy. All patients but two of them showed a reduction in MPXI level. The correlation between Hb and MCV with MPXI has been clearly demonstrated. The therapy in these patients could efficiently increase Hb, normalize MCV, and reduce MPXI. Therefore, confirming MPXI range along with other parameters could demonstrate the efficacy of treatment.

Macrocytosis may be obscured or masked by coexisting iron deficiency, inflammatory diseases or thalassemia minor. On rare occasions, the MCV may be normal, even in the absence of such complicating conditions.[3] Even when masked by a severe microcytic anemia, however, a megaloblastic anemia will usually show hypersegmented neutrophils in the blood and giant metamyelocytes and bands in the marrow, and neutrophil myeloperoxidase levels will be high.[10] Spivak studied six patients with eight episodes of anemia associated with folic acid or vitamin B12 deficiency. They were unaccompanied by macrocytosis but they had hypersegmented neutrophils in all episodes.[11]

CONCLUSION

Elevated MPXI was seen in 82% of our megaloblastic patients. It can be concluded that MPXI measurement

may assist diagnosing of such anemia, being a suitable test in monitoring response to therapy because of significant post-treatmental reduction in 96% of cases. Further investigations should be conducted to assess the specificity of this test in the differential diagnosis of macrocytic states.

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