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ORIGINAL ARTICLE / RESEARCH

Estimation of the Diagnostic Value of Myeloperoxidase Index and Lactate Dehydrogenase in Megaloblastic Anaemia

EIVAZI-ZIAEI J, DASTGIRI S, SANAAT Z

ABSTRACT

Background: Most cases of megaloblastic anaemia corresponded to anaemia with hyper-segmented neutrophils, macro-ovalocytosis and very high serum lactate dehydrogenase (LDH) level. Elevated neutrophil myeloperoxidase index (MPXI) may be indicative of a diagnosis of megaloblastic anaemia.

Study Setting and Design: The aim of this study was to estimate the value of MPXI and LDH in the diagnosis of megaloblastic anaemia to facilitate the diagnosis prior to performing any bone marrow aspirate.

Methods: MPXI and LDH were measured using the first blood sample obtained prior to any transfusion or medical therapy and after therapy in 29 patients diagnosed as megaloblastic anaemia. MPXI was assessed using complete blood count (CBC), performed by Technicon H1 (Bayer) automated cell counter.

Results: Mean value of MPXI significantly decreased after treatment (20.4, CI 95%: 17–23 vs. −0.75, CI 95%: −4 to 2.7, before and after treatment, respectively). The same significant pattern was also observed for LDH (4230, CI 95%: 3096–5369 vs. 783, CI 95%: 492–1075, before and after treatment, respectively). The proportional diagnostic value was significantly higher when both MPXI and LDH were used together in the diagnosis of megaloblastic anaemia (83%, $P < 0.001$), while the same index was 71% ($P < 0.001$) for MPXI and 48% ($P < 0.001$) for LDH, respectively, when they were used alone.

Conclusion: MPXI and LDH values may have a diagnostic role on megaloblastic anaemia. It might be used as a reliable screening tool before doing any other diagnostic procedure.

Key words: Megaloblastic, anaemia, LDH, myeloperoxidase

Introduction

Most cases of megaloblastic anaemia corresponded to a severe macrocytic anaemia with hyper-segmented neutrophils, macro-ovalocytosis and very high serum lactate dehydrogenase (LDH) level [1]. The expected increased LDH activity is the result of an accelerated turnover of bone marrow cells implying the release of this enzyme from dividing and/or decaying cells [2].

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Myeloperoxidase is a microbicidal protein, which is present in the primary granules of myeloid cells and takes part in the defence of the organism [3]. It is synthesised in the promyelocytes where it is packed into azurophilic granules [4]. Technicon H*1 has been used in several studies for the diagnosis of megaloblastic anaemia [5]. Several reports of indicating elevated levels of myeloperoxidase index (MPXI) have recently been reported from different studies [2],[4],[6]. Gulley and colleagues reported the observation of a high neutrophil myeloperoxidase activity (MPXI) in patients with megaloblastic anaemia and

described its simplicity and role in masked megaloblastic anaemia [4].

In this study, we estimated the value of MPXI and LDH in the diagnosis of megaloblastic anaemia to facilitate the diagnosis prior to performing any bone marrow aspirate.

Methods

The medical records of cases diagnosed as megaloblastic anaemia were studied from 2005 to 2006. Twenty-nine cases met the study criteria. The age range for megaloblastic anaemia patients was 12–72 years. Inclusion criteria for megaloblastic anaemia patients included age >12 years, typical peripheral smear, bone marrow aspiration with trephine biopsy findings such as hyper-segmented neutrophils, macro-ovalocytes and giant band cells, who responded to treatment with vitamin B12, 2000 μg subcutaneously or intramuscularly and 1–3 mg oral ingestion of folic acid for 6 weeks. Exclusion criteria were blood transfusion and cases with other diagnosis in trephine biopsy report such as megaloblastoid changes with myelodysplastic syndromes or aplastic anaemia. Peripheral smear and bone marrow aspiration slides were stained with May–Graunwald–Giemsa staining and trephine biopsies stained with haematoxylin and eosin. Two haematologists made comments on peripheral smear and bone marrow slides, while trephine biopsies were assessed by an expert haemato-pathologist. MPXI and LDH were measured using the first blood sample obtained prior to any transfusion or medical therapy performed by Technicon H1 (Bayer) automated cell counter. MPXI is a direct and routine reading parameter in the machine result, with the normal range between −10 and +10. Peroxidase activity and cell size were measured by light absorbance and scatter as each leukocyte flows through a mercury arc light beam [7]. The value was computed as follows: MPXI = mean *X* of sample neutrophil – mean *X* of archetype neutrophil/mean of archetype neutrophil × 100. Mean of sample neutrophil is the average absorption channel (*X*) observed for neutrophils in the sample. Mean of archetype neutrophil is the average absorption channel (*X*) specified for the neutrophil cluster in the normal staining archetype [7]. The values below −25 represented people with myeloperoxidase deficiency and autosomal-recessive anomaly [8]. Serum vitamin B12 and folate levels were determined in some patients. LDH was measured with Hitachi instrument.

Results

The study sample comprised 29 subjects of megaloblastic anaemia studied from 2005 to 2006. The pre- and post-diagnostic characteristics of MPXI and LDH are presented in [Table/Fig 1]. Mean value of MPXI was significantly decreased after treatment (20.4, CI 95%: 17–23 vs. –0.75, CI 95%: –4 to 2.7, before and after treatment, respectively). The same significant pattern was observed for LDH (4230, CI 95%: 3096–5369 vs. 783, CI 95%: 492–1075, before and after treatment, respectively).

Table/Fig 1: Basic characteristics of MPXI and LDH before and after the diagnosis

	MPXI		LDH	
	Before	**After**	**Before**	**After**
Mean	20.4	−0.75	4230	783
Confidence interval 95%	(17–23)	(−4 to 2.7)	(3096–5369)	(492–1075)
Median	22.7	−1.3	3744	521
Standard deviation	8.1	9.2	2981	766
Minimum	4.5	−19.8	625	177
Maximum	34.7	23.7	14,360	3352

Abbreviations: LDH: lactate dehydrogenase; MPXI: myeloperoxidase index.

[Table/Fig 2] and [Table/Fig 3] show the estimation of the proportional diagnostic value (PDV) of MPXI, LDH and both together in the diagnosis of megaloblastic anaemia. The effectiveness of the diagnosis of these methods was compared before and after the treatment, using McNemar statistical test. All study subjects were examined before and after

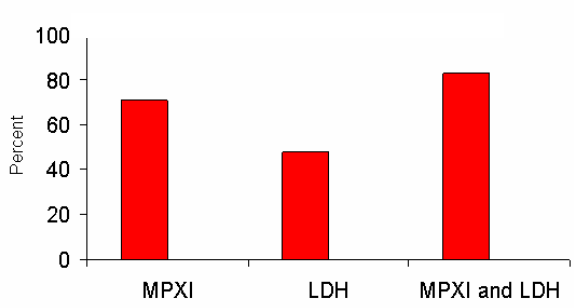
treatment using three indices: first they were diagnosed using MPXI only, second using LDH only and third by MPXI and LDH together. Proportion of patients diagnosed by these three methods was statistically compared before and after treatment. The PDV was significantly higher when both MPXI and LDH were used together in the diagnosis of megaloblastic anaemia (83%, $P < 0.001$), while the same index was 71% ($P < 0.001$) for MPXI and 48% ($P < 0.001$), respectively, for LDH when they were used alone.

Table/Fig 2 Estimation of the proportional diagnostic value (PDV) of MPXI, LDH and both together

		Before		After		Percentage changed (%)	McNemar test (*P*-value)
		n	%	n	%		
MPXI	Abnormal	24	82.8	7	24.1	71	<0.001
LDH	Abnormal	29	100	15	51.7	48	<0.001
MPXI and LDH	Abnormal	24	82.8	4	13.8	83	<0.001

Abbreviations: LDH: lactate dehydrogenase; MPXI: myeloperoxidase index.

Table/Fig 3



Estimation of the proportional diagnostic value (PDV) of MPXI, LDH and both together in 29 patients.

Discussion

This study assessed the diagnostic value of LDH and MPXI in diagnosis of megaloblastic anaemia, suggesting a reliable screening tool before doing bone marrow aspiration and other complicated tests.

Our patients had typical peripheral smear and bone marrow findings with positive response to therapy. We tested B12 and folate levels in only a few patients, and this was a major limitation of this study, although measurement of serum vitamin B12 and serum and red cell folate levels may present problems in interpretation, which must be recognised if diagnostic errors are to be avoided [9]. Another problem of this study is limited number of patients.

The diagnosis of megaloblastic anaemia and the differentiation of folate and vitamin B12 deficiency require, in addition to careful attention to the history and physical findings, the use of laboratory tests [10]. Several tests are usually needed for accurate differential diagnosis of megaloblastic anaemia from other macrocytic anaemias, including peripheral smear and bone marrow studies.

The differential diagnosis of macrocytic anaemia requires reticulocyte counts, vitamin B12 and folate serum levels, thyroid and liver function studies and, in many instances, the assessment of megaloblastic or dysplastic features in bone marrow aspirates [2],[6],[11],[12],[13]. Non-megaloblastic macrocytic anaemia may be accompanied by increased reticulocyte counts (haemolysis and haemorrhage) or by normal or decreased reticulocyte counts (alcoholism, liver disease, hypothyroidism and various bone marrow disorders) [12].

LDH increases in haemolytic anaemia, ischaemic heart diseases, and liver and muscle abnormalities [14]. But according to clinical and physical findings, we can rule out many conditions with these disorders. However, the important problem here is differentiation of treatable diseases (i.e. megaloblastic anaemia) from other serious ones.

Autoimmune thrombocytopenia may be seen in rare cases with haemolysis resembling megaloblastic anaemia with increased unconjugated bilirubin and LDH, but haemoglobinuria and haemosiderinuria and increased reticulocyte count can differentiate it from megaloblastic anaemia, which has low reticulocyte count [8]. Jaswal and colleagues evaluated the efficacy of total serum LDH levels and LDH isoenzyme pattern in the diagnosis of megaloblastic anaemia [15]. Acute leukaemia is another condition in differential diagnosis with

megaloblastic anaemia, but clinical findings such as splenomegaly, adenopathy and poor general condition can help in diagnosis. On the other hand, MPXI in leukaemia is lower than megaloblastic anaemia. According to Tsakona et al., mean peroxidase activity value was −12.6 in acute myeloid vs. −0.6, $P < 0.01$ in acute lymphoblastic leukaemia [16]. Bone marrow findings suggestive of severe megaloblastic anaemia can also be present in myelodysplasia [17],[18].

Although, MPXI has not been studied in myelodysplastic syndrome, but, according to Davey et al., neutrophils and bands from patients with acute myeloid leukaemia and myelodysplastic syndrome have a defect in one or more of the constituents of primary and/or secondary granules and have exhibited a qualitative and/or quantitative deficiency in myeloperoxidase, so we can suspect the same changes in MPXI in myelodysplastic syndrome [19]. Also a secondary form of myeloperoxidase deficiency due to discrete chromosomal rearrangements with gene disruption has been reported in some patients with acute myeloid leukaemia and myelodysplastic syndrome [20].

If the patient has multiple deficiencies like iron deficiency coexisting with B12 and folate deficiency, the typical features may not be evident and diagnosis becomes difficult [21],[22].

MPXI measurement may be particularly useful in identifying cases of 'masked megaloblastic anaemia' where the MCV is below 100 fl. The advantage of the MPXI over other methods of uncovering masked megaloblastic anaemia is its simplicity when performed as part of a routine complete blood count (CBC) on an automated haematology instrument [4].

Conclusion

We concluded that MPXI and LDH measurement can be used as a screening test for diagnosis of megaloblastic anaemia before performing bone marrow aspiration. Further studies are required for differentiation of another macrocytic anaemia by using MPXI.

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