Original & Research

Evaluation of Macrocytosis in a Routine Haemogram

Paresh Marathe, Ketki Karne-Choksi, Nina Desai, Shilpa Ramasamy

Abstract

Macrocytosis is a common laboratory abnormality seen in various clinical settings. The reported prevalence across various studies range from 1.7-4 %.[4-9] Macrocytosis precedes anaemia and can occur without anaemia. Mild macrocytosis with Mean Corpuscular Volume (MCV) between 100-110 femtolitres(fl) is more common than severe macrocytosis and can remain unexplained even after extensive evaluation.[8] Macrocytic anaemia is classified into two groups-Megaloblastic and non-megaloblastic anaemia depending upon the morphological and biochemical finding.

We conducted this study to find out the underlying aetiologies in patients with macrocytosis. We also evaluated various clinical, biochemical and haematological features in these patients so as to find out the difference between megaloblastic macrocytic and non-megaloblastic macrocytic anaemia. This has important clinical implications as assays of serum B_{12} and folate levels may not be available in all the centres, especially in resource-poor settings and also these assays may not be accurate. Hence any finding of macrocytosis should be thoroughly evaluated as it can precede anaemia, or symptoms of underlying disease process, thereby helping in early diagnosis and treatment.

Introduction

Anaemia is one of the most common public health problems across all the age groups worldwide. WHO defines anaemia as haemoglobin < 13 gm% in males (>15 years), < 12 gm% in non-pregnant females and < 11 gm% in pregnant females.[1] Anaemia can be classified in many ways, depending upon the cause of anaemia, mechanism of anaemia, RBC indices etc. Wintrobe first classified anaemia into 4 groups, namely macrocytic, microcytic, normocytic and microcytic hypochromic depending on RBC indices.[2]

The normal Mean Corpuscular Volume (MCV) in adults ranges from 80 to l00 femtolitres(fl). Macrocytosis is defined as MCV >100 fl. Macrocytosis is a common laboratory abnormality. The prevalence of macrocytosis varies from 7-4% across various studies.[4,5,6]

Macrocytosis can be asymptomatic, present with anaemic symptoms or symptoms of underlying disease. The significance of macrocytosis can be underestimated, as up to 60% of patients may not have associated anaemia unless accompanied by other abnormalities.[7]

Macrocytosis by itself does not cause any symptoms or complications. However, its presence can give clue regarding the presence of an underlying disease, thereby helping in early diagnosis and treatment. Hence, presence of macrocytosis needs clinical and laboratory assessment to

Dept. of Pathology, Bombay Hospital Institute of Medical Sciences, 12, New Marine Lines, Mumbai - 400 020.

identify the underlying cause.

The causes of macrocytosis are heterogeneous and the mechanisms causing it are diverse.

Macrocytosis is usually further sub classified into megaloblastic and non-megaloblastic anaemia depending upon the underlying pathogenesis and bone marrow findings. The various aetiologies of macrocytosis include vitamin B_{12} and/or folic acid deficiency (megaloblastic anaemia), primary bone marrow disorders like acute leukaemia, aplastic anaemia, multiple myeloma, myelodysplastic syndromes, chronic alcoholism, alcoholic and non-alcoholic liver disorder, hypothyroidism, chronic drug intake (methotrexate, hydroxyurea, valproate, zidovudine, metformin etc).

Aims and Objectives

1. To identify causes of macrocytosis in a routine haemogram.
2. To evaluate haematological features in macrocytosis of different aetiologies.

Material and Methods

This was a prospective, observational study conducted in the department of Pathology, Bombay Hospital and Research Centre from August 2015 to March 2018.

Patients referred to the pathology department for CBC and peripheral smear were screened for the presence of macrocytosis and 100 consecutive cases were included in the study.

- Inclusion criteria

All adult patients (>18 years) with MCV > 100 fl with anaemia, defined as haemoglobin (Hb) of

1. < 13 g/dl in male.
2. <12 g/dl in female.

- Exclusion criteria

1. Newborns
2. Pregnant women
3. Reticulocytosis
4. Spurious macrocytosis (cold agglutination, hyperglycaemia, leucocytosis, delayed sample processing.

Other investigations done on these patients as per the discretion of the treating physician was also noted.

- Methods

1. CBC was analysed on fully automated five part blood cell counter.
2. Manual peripheral smear examination was done for counter checking in every case after staining the blood smear with Wright's stain.
3. Whenever indicated bone marrow aspiration and biopsy were performed on a case to case basis from posterior superior iliac spine using Salah bone marrow aspiration needle and Jamshidi bone marrow biopsy needle.

The bone marrow smears were air dried and stained by Wright stain. Imprints were made from the biopsy, air dried and stained by Wright's stain.

The bone marrow biopsy sample was fixed with 10% buffered formalin. It was then processed, sliced and stained by Haematoxylin and Eosin stain.

4. Serum vitamin B_{12} assay was done using Architect Plus i1000 SR.
5. Seum folate was done using Cobas e411 of Rosche.

- In all the patients with macrocytosis, a thorough history and complete physical examination was performed. Details of the symptoms, dietary

history, surgery, alcohol addiction and presence of other medical or surgical diseases like diabetes mellitus, hypothyroidism, gastrectomy etc were carefully noted.

- Complete haemogram (included Haemoglobin concentration, red cell indices, total and differential white cell count, platelet count, reticulocyte count and red cell distribution width) and peripheral blood smear was available for all the patients.
- The serum vitamin B_{12} was done in all 100 cases. Serum folate levels were done in only 9 patients.
- Vitamin B1; deficiency was considered when patient had serum vitamin B_{12} < 187 picogram/ml (reference range 187-883 pg/ml).
- Folate deficiency was considered when serum folate levels were < 3 ng/ml (reference range 3-17 ng/ml). Megaloblastic anaemia was considered when there was either serum vitamin B_{12}/folate deficiency or both. Chronic alcoholism was labelled when patient had history of persistent alcohol intake, alcohol tolerance or withdrawal symptoms.
- Thyroid, Liver function tests, Fasting blood sugars and Post prandial blood sugars were performed for all patients.

- Statistical tests

For quantitative data mean, minimum and maximum were calculated. Standard deviation of these quantitative data was calculated. The comparison between mean of two quantitative variables and identification of their significance was done using unpaired 't' test (student's 't' test). A p value of less than 0.05 was considered statistically significant.

Results

A total of 100 patients with macrocytosis were evaluated.

- Age wise distribution

Mean age was- 49.47 years, Minimum age-20 years, Maximum age was- 87 years

51-60 years age group-27 patients (Majority)

41-50 years age group -25 patients

- Gender wise distribution

There were 80 males and 20 females

- Aetiologic spectrum

The distribution of the patients as per the aetiology is shown in Table 2. Megaloblastic anaemia was the most common cause of macrocytosis (39 patients) followed by liver disease and primary bone marrow disorder.

Table 2

No	Aetiology	Number of patients (n= 100)
1	Megalo blastic anaemia	39
a.	Vitamin B_{12} deficiency	30
b.	Vitamin B_{12} deficiency + Folate deficiency	4
c.	Drug induced	5
I	Hydoxyurea	1
Ii	Methotrexate	1
Iii	Metformin	1
Iv	Sulphasalazine	1
V	Zidovudine	1
2	Liver disease	20
A	Alcohol related liver diseases	11
B	Non-alcohol related liver diseases	9
3	Primary bone marrow disorders	18
A	Myelodysplastic syndrome (MDS)	6
B	Aplastic anaemia (AA)	6
C	Acute leukaemia (AL)	4
D	Multiple Myeloma (MM)	2
4	Chronic alcoholism	10
5	Hipothyroidism	5
6	Combined	6
A	Megaloblastic anaemia +liver disease	2
B	Megaloblastic anaemia + hypothyroidism	2
C	Liver disease +Drug (Zidovudine)	1
D	Hypothyroidism+Aplastic anaemia	1
7	Chronic kidney disease	2

- Diet in megaloblastic anaemia
  Majority of the patients -31 out of 39 (79%) with megaloblastic anaemia were vegetarian and the remaining 21% were non vegetarian.
- Clinical Features
  - The most common symptoms were related to anaemia like weakness. fatigability and dyspnoea, seen in 79 patients.
  - Others were pedal oedema (42). Jaundice (21), Skin changes (23), Neuropsychiatric symptoms (12) like tingling numbness, irritability, imbalance etc, bleeding manifestations (13), Abdominal distension (22).
- Clinical signs
  - The most common sign was pallor corroborating with the most common symptom of anaemia seen in 79 patients.
  - Others were lcterus (21), Skin abnormality (29), Glossitis & cheilitis (53), Hepatomegaly (25), Splenomegaly (33), CNS abnormality (6)
- Peripheral blood smear
  - The important feature in the peripheral blood smear of patients with macrocytosis includes hypersegmented neutrophils and macroovalocytes.
  - They were found in 38 out of 100 patients with macrocytosis and in 35 megaloblastic patients (90%).
- Comparison of haematological parameters between megaloblastic and non-megaloblastic group
  - The mean haemoglobin in the megaloblastic group was significantly lower than the non-megaloblastic group.
  - The MCV, MCH, LDH were significantly higher in the megaloblastic group as compared to non-megaloblastic group. RDW was higher in the megaloblastic group as compared to non-megaloblastic group.
  - The mean serum Vitamin B_{12} level in the megaloblastic group was 136.39 ± 108.2. This was significantly lower in the megaloblastic group as compared to the non-megaloblastic group.
  - MG-Megaloblastic, NMG- Non-megaloblastic, CC-Combined cause, P value calculated by unpaired 't' test

Table 3

No	Feature	MG (n=39)		NMG (n=55)		CC (n=6)	
		Mean	SD	Mean	SD	Mean	P value
1	Hb (g %)	8.95	2.42	10.12	2.62	7.46	0.029
2	RBC count (mill/cumm)	2.82	0.97	3.12	0.93	2.04	0.13
3	MCV (fl)	115.57	6.05	105.24	4.32	112.4	0.0001
4	MCH (pg)	36.35	3.33	34.01	1.91	35.98	0.0001
5	MCHC (g/dl)	32.42	1.96	32.42	0.84	32.04	0.99
6	PCV (%)	31.99	10.98	32.61	9.73	23.08	0.765
7	TLC (/cumm)	6194.87	2703.4	6379.82	2858.89	7940	0.75
8	Platelet count (/cumm)	190725.64	96311	147786	86885	83200	0.025
9	RDW (%)	18.49	4.88	17.78	3.62	23.8	0.42
10	LDH (U/l)	1125.71	645.24	233.87	170.08	2105	0.0001
11	Vitamin B_{12} (ng/dl)	136.39	108.2	526.86	398.22	203.2	0.0001

MG- Megaloblastic, NMG-Non-megaloblastic, CC-Combined cause, P value calculated by unpaired 't' test

Discussion

1. Age: Majority of the patients belonged

to the age group of 40 to 60 years(5l %). This finding was similar to the study by McPhedran and Davidson[4,5]

2. Gender: Unlike iron deficiency anaemia, which has female preponderance, macrocytic anaemia does not have any sex predilection. There was male preponderance in our study.
3. Aetiology : The results from our study showed that Megaloblastic anaemia was the most common cause of macrocytosis (39 patients) followed by liver disease and primary bone marrow disorder. The aetiologic spectrum of macrocytosis seen in our study is similar to that of Wintrobe and McPhedran.
4. Dietary pattern: The dietary pattern revealed that 79% patients with megaloblastic anaemia were vegetarian. This is expected, as vegetarian diet is known to be deficient in Vitamin B_{12}. In our study there were 6 patients in the non-vegetarian group. Of this 1 patient was evaluated and found to be a case of pernicious anaemia. Rest 5 were not evaluated for pernicious anaemia due to financial constraints. Also 2 patients from this group had drug-induced macrocytosis which was responsible for megaloblastic anaemia.
5. Clinical features: The most common sign (pallor) and symptom (weakness, fatigue, dyspnoea) were related to anaemia. Anaemia was seen in 79 % of patients and hence signs and symptoms pertaining to anaemia were the most common feature.
6. Comparison of laboratory parameters between megaloblastic and non-megaloblastic anaemia

- Vineeta U and A Kannan from India conducted a study to compare the 2 groups. They found that mean haemoglobin in the megaloblastic group was lower than the non-megaloblastic group and the MCV was higher in the megaloblastic group.[10] This finding is similar to our study.
- Serum LDH was higher in the megaloblastic group and this was statistically significant. A Kannan in her study found similar results.[10]
- RDW was higher in the megaloblastic group RDW reflects the degree of anisocytosis, which is more in the megaloblastic group. It was not statistically significant (P value-0.42). This is probably because patients in our group did not have severe anaemia (Hb < 8 gm %).
- The mean serum Vitamin B_{12} level in the megaloblastic group was 136.39 ± 108.2. This was significantly lower in the megaloblastic group as compared to the non-megaloblastic group. A study by Yadav MK showed that in 80% of megaloblastic patients had lower serum Vitamin B_{12} levels.[11]

7. Peripheral blood smear - The peripheral blood smear revealed hypersegmented neutrophils and macroovalocytes in 90% of cases with megaloblastic anaemia. Hypersegmented neutrophils (60%) and macro-ovalocytes (100%) were seen in study conducted by A

Kannan.[10]

Conclusions

1. Macrocytosis is a common haematological problem.
2. Megaloblastic anaemia due to vitamin B_{12} deficiency, liver diseases, bone marrow disorders, chronic alcoholism are common causes.
3. The MCV, MCH, LDH are significantly higher in the megaloblastic group as compared to non-megaloblastic group, helpful to differentiate between the two
4. Identification of macrocytosis and its aetiology helps in early recognition of disease process and prompt treatment, which is likely to improve the patient outcomes.

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