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CORRELATION OF SERUM VITAMIN B_{12} WITH PERIPHERAL SMEAR FINDINGS OF MEGALOBLASTIC ANEMIA AND ITS ASSOCIATION WITH BONE MARROW FINDINGS

Gurunani Reshma[1], Shrivastav Atul[2], Maru Alpesh[3], Choksi Tejas[4], Trivedi Naresh[5],Agnihotri Ashok[6]

1.DCP, Resident, Dept of Pathology, C. U. Shah Medical College, Surendranagar.
2. MD, DNB, Asst. prof., Dept of Pathology, C. U. Shah Medical College, Surendranagar.
3. MD, Resident, Dept of Pathology, C. U. Shah Medical College, Surendranagar.
4. MD, Professor, Dept of Pathology, C. U. Shah Medical College, Surendranagar.
5. MD, Sr. Professor, Dept of Pathology, C. U. Shah Medical College, Surendranagar.
6. MD, Sr. Professor & Head of Dept of Pathology, C. U. Shah Medical College, Surendranagar.

ABSTRACT:

Introduction: Vitamin B12 deficiency is known to cause Megaloblastic anaemia. The objective of this study is to correlate Serum Vitamin B12 levels with peripheral smear findings of Macrocytosis, Macroovalocytosis and Hypersegmented neutrophils which are considered to be characteristic peripheral smear findings of Megaloblastic anaemia and its association with bone marrow findings of megaloblasticanaemia.

Material & Methods: In this retrospective study, laboratory records of 200 patients were collected along with 20 normal healthy individuals as controls. Serum vitamin B12 levels and peripheral smear findings were compared in each patient.

Results: Out of 200 patients 24(12%) had mild deficiency 88(44%) had moderate deficiency, and 88(44%) had severe deficiency.

Conclusion: Serum vitamin B12 levels were well correlated with peripheral smear findings of macrocytosis, macroovalocytosis, and hypersegmented neutrophils.

Key words: Macrocytosis, macroovalocytosis, hypersegmented neutrophils, vitamin B12.

INTRODUCTION:

Macrocytosis, defined as a mean corpuscular volume greater than 100 fl, occurs in approximately 3 percent of the general population[1].The most common etiologies are alcoholism, vitamin B_{12} and folate deficiencies, and medications[2]. History and physical examination, vitamin B_{12} level, reticulocyte count, and a peripheral smear are helpful in delineating the underlying cause of macrocytosis. It has Megaloblastic and non Megaloblastic causes. Megaloblastic anemia includes a group of disorders characterized by one or more peripheral cytopenias, oval macrocytosis, iron overload, erythroid hyperplasia[3].It is commonly caused by a Deficiency of

*Corresponding Author Address: Dr. AtulShrivastav, Department of Pathology, C. U. Shah Medical College, Dudhrej Road, Surendranagar, Gujarat, India. 363001 E-mail: atulshri@ymail.com

vitamin B_{12} or folate deficiency resulting in impairment of DNA synthesis[4].When the peripheral smear is nonmegaloblastic, the reticulocyte count helps differentiate between drug or alcohol toxicity and hemolysis or hemorrhage, hypothyroidism, liver disease, and primary bone marrow dysplasia (including myelodysplasia and myeloproliferative disorders) are some other common causes.

Vitamin B_{12} deficiency is thought to be more common than was previously believed. In clinical practice, the finding of an elevated mean corpuscular volume (MCV), macrocytic anaemia or specific neurological symptoms are commonly used by physicians as an indicator for Megaloblasticanaemia caused by vitamin B_{12} deficiency. We evaluated the clinical profiles of our patients with vitamin B_{12} deficiency and tried to ascertain how useful MCV and the peripheral smear were in diagnosis and whether the correlation with severity of deficiency was significant to exclude the need of invasive procedure of bone marrow examination.

Aims and objectives:

- To find out correlation of severity of vitamin B_{12} deficiency with the peripheral smear findings of megaloblastic anemia.
- To identify the need of bone marrow aspiration to confirm the diagnosis of Megaloblasticanaemia.
- Thereby, this study would help in planning the diagnostic & therapeutic approach in patients with megaloblastic anemia and implementing the invasive procedures like bone marrow aspiration only when necessary.

MATERIAL AND METHODS:

This cross sectional prospective study was carried out from July 2012 to August 2013 at Central Clinical Laboratory of Department of pathology at C. U. Shah Medical College and Hospital, Surendranagar. This study included 200 patients of both sexes. Case selection was based on clinical features and supported by laboratory evidence.

Source of data: patients were recruited from all clinical departments, mainly from medicine department of C. U. Shah medical college and hospital. Prior permission from institutional ethical committee is taken.

Method of collection of data: Detailed clinical history is taken and thorough physical examination was performed in each case. Complete blood count by automated blood cell counters, peripheral smears, serum vitamin B_{12} levels by automated biochemistry analyzers are done and bone marrow aspiration/biopsy were performed wherever possible.

Data pertaining to serum vitamin B12 levels (Normal:190-660 pg/ml) and peripheral smear findings were compared.

Inclusion criteria in our study were as follows:

- Patients having clinical features suggestive of vitamin B_{12} deficiency.
- Serum vitamin B_{12} levels < 190 pg/ml.

Exclusion criteria in our study were as follows:

- Patients who had normal vitamin B_{12} levels.
- Patients who had taken empirical vitamin B_{12} treatment, oral or injectable.
- Patients having associated iron deficiency.

Blood samples of patients were obtained by routine phlebotomy procedure. Written consent was taken from all patients for using their sample for research purpose. About 4 ml of blood sample was collected 2 mL in EDTA vacutte and 2 mL in plain vacute. EDTA anticoagulated blood is processed through automated hematology analyzer – Beckman coulter AcTdiff2 and hematological parameters were obtained, out of which hemoglobin, mean corpuscular volume (MCV) were included in this study.

Serum from Plain vacutte is used for vitamin B_{12} analysis which was done by automated biochemistry analyzer – Siemens dimension system. All patients were divided into mild, moderate and severe deficiency category according to their vitamin B_{12} levels. (mild: 150-189, moderate:100-149, severe: <100).Patients with serum vitamin B_{12} levels between 150-189 pg/ml were included in mild deficiency, those between 100-149 pg/ml were included in moderate deficiency and patients with vitamin B_{12} levels <100 pg/ml were included in severe deficiency.

Peripheral smear was stained by Leishman stain for all the cases and examined in detail and patients were distributed according to their smear findings into having macrocytosis, macro-ovalocytosis with/without hyper segmented neutrophils. Bone marrow aspiration was done after obtaining written consent from the patient or guardian.Bone marrow sample obtained by routine bone marrow aspiration procedures by salah's needle. Staining of bone marrow aspirate was done with Leishman stain.

RESULTS:

Out of a total of 200 patients who were recruited in the study, 122 were male and 78 were female. The age range of the patients was 13–86 years. The peak incidence was in age group 45-55 years. The mean age was 42.44 years. Most of the Patients presented with clinical features pertaining to anemia. 41% patients had generalized body weakness, 20.5% patients had fever, 38.5% patients had anorexia and out of all, 79 patients had associated pallor. All other observations were written in tabulated and figure form (Tables 1 – 2, Figure 1 &2).Significance of all data was checked statistically by chi square method which was found significant.

Peripheral Blood Smear findings: In most cases there is a macrocytic anaemia with oval macrocytes being particularly characteristic. Some degree of anisocytosis and poikilocytosis is usual and when anaemia is severe there are striking morphological abnormalities including the presence of tear drop cells, basophilic stippling, occasional Howell jolly bodies and circulating megaloblasts. Hypersegmented neutrophils are usually present; they are highly suggestive of megaloblastic erythropoiesis although not pathognomic. In severe Megaloblastic anaemia leucopenia and thrombocytopenia also occur[5] (figure 1-4). However, some patients with low vitamin b12 levels were found to be having normal hemoglobin levels and indices and also normal peripheral smear. Such patients were excluded from study.

Bone marrow aspiration findings: Out of these 200 patients, 24 patients gave consent for Bone Marrow aspiration procedure and were diagnosed as Megaloblastic anaemia.The bone marrow is hypercellular. Erythropoiesis is hyperplastic and characterized by the presence of megaloblasts.These are large cells with a chromatin pattern more primitive than is appropriate for the degree of maturation of cytoplasm. Giant metamyelocytes & Band cells are usually present. They are twice to three times the size of a normal metamyelocyte. When megaloblastic features in erythroblasts are partly or largely masked by co-existing iron deficiency; then detection of giant metamyelocytes are diagnostically important because they are consistently present in the smears[5].

Bone marrow biopsy: There is variable hypercellularity with loss of fat cells. In some cases this can be so severe that it may resemble the 'packed marrow' appearance seen in acute leukaemia on examination at low power. There is erythroid hyperplasia with predominance of immature precursors. The early erythroid cells have large, round to oval nuclei with one or more basophilic nucleoli which often appear to have rather irregular margins on the nuclear membrane. There is usually a moderate amount of intensely basophilic cytoplasm. Small Golgi zones may be seen. Giant metamyelocytes are usually easily seen. Megakaryocyte numbers may be normal or decreased[3].

DISCUSSION:

Megaloblastic anemia is usually consequent of deficiency of vitamin B_{12}.Some patients with acute myeloid leukemia (AML) or Myelodysplastic syndrome (MDS) also have megaloblastic erythropoiesis[6].However, if typical peripheral blood features of megaloblastic erythropoiesis are lacking or if atypical features are present, bone marrow aspiration should be performed. Further tests indicated in patients with Megaloblastic anaemia are assays of serum vitamin B_{12} followed when appropriate, by tests for autoantibodies and a schilling test[3].

The term macrocytosis refers to a blood condition in which red blood cells (RBC)

are larger than normal. Macrocytosis is reported in terms of mean corpuscular volume (MCV). Normal MCV values range from 80 to 100 femtoliters (fl) and vary by age and reference laboratory[6].

Macrocytosis of red blood cells is an early change and increases progressively in megaloblastic anaemia. Individual macrocytes appear first, followed by a gradual rise in mean corpuscular volume (MCV) that eventually crosses the line into abnormality (>97 fl), long before the haemoglobin levels fall.[4] In the case of cobalamin deficiency, with its slow progression, macrocytosis precedes anaemia by months[7]. Macro-ovalocytes are especially characteristic of megaloblastic anaemia but are not specific. Early megaloblastic changes in the bone marrow precede the macrocytosis but tend to be mild and easily missed.

Macrocytosis can be identified by reviewing peripheral blood smears and/or by automated RBC indices. The peripheral blood smear is more sensitive than RBC indices for identifying early macrocytic changes because the MCV represents the mean of the distribution curve and is insensitive to the presence of small numbers of macrocytes[8].Although determination of the MCV by automated blood cell counter is rarely inaccurate,partial occlusion of the instrument aperture and/or leaving the blood sample at room temperature for several hours may also result in false elevations of the MCV value as suggested by Lawrence AC et al[9]. During our study similar results were found and such samples were not included in study.

In patients with elevated MCV values, laboratory tests for vitamin B_{12} deficiencies are routinely ordered by physicians, although these tests are limited by their low sensitivity and specificity as investigated by Davidson RJ et al[10] in study on high MCV. In our study MCV values correlates with low vitamin B_{12} levels. In another study done by Florence asliniaet al[11] macroovalocytosis was associated with MCV of more than 115 fl, while in our study it was found to be lower.

In our study the peripheral smear findings of macrocytosis, macroovalocytosis and / or hypersegmented neutrophils were all taken into account separately to identify whether this findings correlate with severity of vitamin B_{12} deficiency in patients of megaloblastic anaemia. Here we observed that on peripheral smear, macrocytosis and macroovalocytosis correlates with severity of vitamin B_{12} deficiency which was similar to the findings of study done by Mwanda OW et al[12] who proposed that a model consisting of oval macrocytosis on blood film and megaloblastic bone marrow can be treated with vitamin B_{12} injections considering it as vitamin B_{12} deficiency in settings where vitamin B_{12} serum level assays are not easily available.

While hypersegmented neutrophils along with these two features did not correlate with severity of deficiency but was a consistent finding in all cases. This findings were similar to a study done by

Nath BJ et al[13]. it was observed that MCV singley is not sufficient for screening of B_{12} deficiency; examination of the blood smear for hypersegmentation is essential as it is more sensitive than MCV. But in our study Mean corpuscular volume though did not correlate with severity of deficiency but was consistently found to be increased than normal levels in patients of vitamin B_{12} deficiency

CONCLUSION:

After consideration of findings of our study and its comparison with other similar studies done in previous years. we came at the conclusion that serum vitamin B_{12} levels correlates with macrocytosis and macroovalocytosis in deficiency state. Although hypersegmented neutrophils did not correlate well with severity of deficiency of B_{12} but it is suggestive of low vitamin B_{12} levels. An elevated MCV justifies the measurement of serum B_{12} but it should be supported by peripheral smear findings of macrocytosis, macroovalocytosis and hypersegmented neutrophils and if these findings are associated with clinical features of B_{12} deficiency then patients can be empirically treated by vitamin B_{12}. Low vitamin B_{12} levels along with peripheral smear findings of megaloblasticanaemia, rules out the need for invasive procedure of bone marrow examination for which usually patients refuse to give consent.

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TABLES:

TABLE 1: shows different parameters of patients of Megaloblastic anemia (n=200).

Parameter	Mean ± Standard Distribution	Range
1) Hb (g/dL)	7.3 ± 1.84	2.2-11.8
2) RBC Count (x10^{12} /L)	4.79 ± 0.67	3.35-5.67
3)MCV(Mean Corpuscular Volume) (fL)	107.38 ± 12.53	96.9 – 136.3
4) MCH (pg) (Mean Corpuscular Hemoglobin)	25.38 ± 4.32	15.6-35.3
5) RDW (%) (Red cell Distribution Width)	15.41 ± 2.46	12.9-18.3
6) Serum Vitamin B12 levels (pg/ml	111.76 ± 38.56	60.1 –182.9

TABLE 2: distribution of patients according to severity of vitamin b_{12}deficiency

Severity of vitamin B_{12} deficiency	No. of patients	Percentage
Mild	24	12
Moderate	88	44
Severe	88	44

FIGURES:

Figure 1: Showsdistribution of patients according to age group.

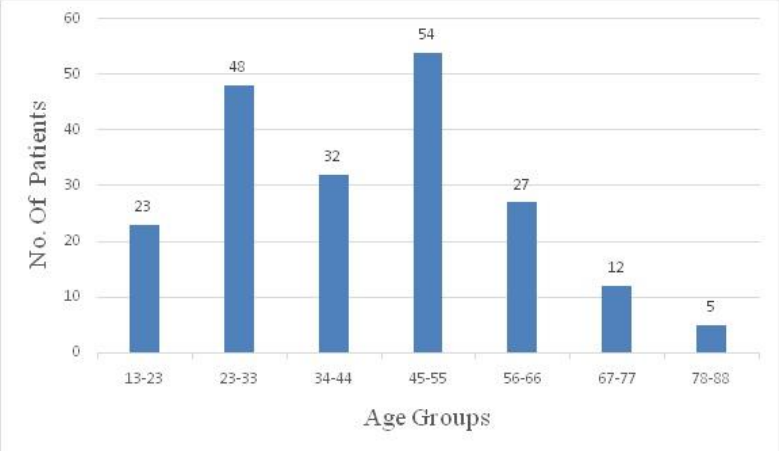

Figure 2: shows distribution of patients according to peripheral smear findings.