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Original Research Article

Comparative Analysis of Bone Marrow Cellularity in Patients with Nutritional and Non-Nutritional Anemia

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Abstract

Background: Anemia is a frequent hematological condition which can be due to nutritional deficiency, chronic diseases, renal diseases, hemolytic diseases, and the like. Bone marrow aspiration will give helpful information on marrow activity and marrow cellularity and may help determine the cause of anemia. The aim of the present study was to compare the bone marrow cellularity in cases of nutritional and non-nutritional anemia.

Objective: To outline the distribution of the cellularity of the bone marrow and related morphological changes in patients with nutritional and non-nutritional anemia.

Materials and Methods: The study was a comparative observation study carried out for 1 year. Fifty-eight patients were diagnosed as anemia and included. The study population comprised of 40 nutritional anaemic patients and 40 non-nutritional anaemic patients. Complete blood count, peripheral blood smears, biochemical investigations and bone marrow aspirates were analyzed. The cellularity of the bone marrow was categorised as hypocellular, normocellular or hypercellular and erythroid and myeloid activity were evaluated and other abnormalities were identified.

Results: Nutritional anemia was found in 40 patients (50%) while non-nutritional anemia was found in 40 (50%) of total 80 patients. Iron deficiency anemia was the most common nutritional anemia. Twenty-six (65%) patients with nutritional anemia had hypercellular bone marrow while 12 (30%) patients with non-nutritional anemia had hypercellular bone marrow. In 27.5% and 45% of the patients, respectively, normocellular marrow was observed, and in 7.5% and 25% of the patients, respectively, hypocellular marrow was observed. More common in nutritional anemia was erythroid hyperplasia. Vitamin B12 deficiency and folate deficiency were the predominant megaloblastic disorders seen. Marrow cellularity was different between the two groups and is statistically significant.

Conclusion: There is a difference in the pattern of bone marrow cellularity in nutritional and non-nutritional anemia. The hypercellular marrow was more commonly associated with nutritional anemia, and the erythroid hyperplasia with nutritional anemia and a more variable pattern with non-nutritional anemia. The bone marrow examination, when clinically indicated, will be useful when complemented by peripheral blood and biochemical results.

Keywords: Anemia, Nutritional Anemia, Non-Nutritional Anemia, Bone Marrow, Cellularity, Erythroid Hyperplasia, Megaloblastic Anemia, Iron Deficiency.

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Introduction

Anemia is one of the most frequently seen haematological diseases in clinical practice, and it is an important public health issue globally. It is defined as having a lower level of haemoglobin than the normal level needed to satisfy the tissue oxygen needs [1]. Anemia can be caused by blood loss, destruction of red blood cells or by a failure of the body to produce enough red blood cells. There are many different causes of anemia, ranging from nutritional deficiencies, chronic diseases, renal disorders, hemolytic conditions, infections, endocrine disorders to primary disorders of the bone marrow [2]. "Understanding the etiology is important since clinical management and prognosis are widely variable. An important type of anemia is nutritional anemia and it is primarily associated with inadequate intake of the nutrients essential for the normal production of red blood cells. The prime

nutrients involved in erythropoiesis are iron, vitamin B12 and folate. Iron is required for hemoglobin synthesis, while vitamin B12 and folate are needed for the normal processes of DNA synthesis and maturation of the hematopoietic cells [3]. Iron deficiency is a common nutritional cause of anemia, typically and almost exclusively resulting in a microcytic hypochromic pattern in peripheral blood. In cases of Vitamin B12 and folate deficiencies, DNA synthesis is affected, and effective hematopoiesis does not take place, causing macrocytic or megaloblastic anemia [4]. Deficiencies can occur in isolation and/or in combination, and result in a range of hematological and morphological abnormalities.

Bone marrow examination is a direct method of obtaining information on the activity of the marrow, and is an important investigation in selected cases with unexplained anemia, abnormal peripheral blood findings, or suspected bone marrow disease [5]. Bone marrow aspirate can be used to evaluate cellularity, erythroid and myeloid precursors, megakaryocytes, maturation pattern and abnormal cells. Bone marrow cellularity is the ratio of fat cells to the cells in bone marrow. It usually changes as a personage and thus should be evaluated in the context of age. Depending on the overall cell content of the marrow, this can be termed hypocellular, normocellular or hypercellular [6]. Characteristic changes in bone marrow cellularity and morphology may be produced by nutritional deficiencies. Erythroid hyperplasia may accompany iron deficiency due to the attempt by the marrow to catch up with the production of hemoglobin. Evaluation of marrow iron stores can also aid in the diagnosis of iron deficiency when laboratory results are equivocal [7]. In B12 and folate deficiency, DNA synthesis is impaired leading to megaloblastic changes. Marrow is usually hypercellular, due to increased hematopoiesis, mainly erythroid, with ineffective production of blood cells. Megaloblastic changes may also affect the granulocytic series, resulting in abnormalities in cellular maturation [8].

Non-nutritional anemia is a general term for all cases of anemia that are not caused by a nutritional deficiency. Anemia due to chronic disease and inflammation, chronic kidney disease, hemolytic disorders, infections, endocrine disorders and some conditions of the marrow. The bone marrow reaction within these diseases may differ according to the mechanism [9]. Some conditions may have an increased erythropoietic activity as a compensatory response while others may have a decreased hematopoietic activity and hypocellular marrow. Hence, unlike nutritional anemia the non-nutritional anemia does not show a single characteristic pattern of marrow cellularity. The peripheral blood findings and bone marrow morphology have important relationships that will facilitate understanding of the pathogenesis of anemia [10]. The hemoglobin level, red blood cell indices, peripheral smear morphology, reticulocyte response, iron profile, the level of vitamin B12 and folate, and other biochemical investigations are important tools for determining the cause of anemia. Bone marrow aspiration may help clarify the diagnosis in some patients in whom the clinical and laboratory data are not enough to establish the diagnosis or to explain the degree or type of anemia. Bone marrow cellularity must be analysed with care as it can be affected by age, quality of the sample, underlying systemic illness and previous therapy [11].

There are a number of studies that have examined the bone marrow morphology in nutritional anaemias, especially in iron deficiency and megaloblastic anaemia. Nutritional deficiency has been shown to cause alterations in the cellularity of the marrow, in erythroid activity, maturation and iron stores. In patients with pancytopenia, other studies have examined the bone marrow in patients with various non-nutrient diseases. Comparatively, however, fewer studies have examined the differences in total bone marrow cellularity in both nutritional and non-nutritional anemias in the same study population.

A comparison of the marrow cellularity of one patient with another may be useful in determining if marrow cellularity is different and thereby help in understanding the significance of the difference in the context of the evaluation of anemia [12]. So, the present study emphasizes the comparative study of bone marrow cellularity in patients with nutritional and non-nutritional anemia. Patients will be classified as either a nutritional or non-nutritional group by clinical, haematological and relevant biochemical parameters.

The findings of bone marrow aspiration will be analysed in terms of the over-all cellularity, erythroid activity, myeloid activity and the associated morphological abnormalities. The study will also determine if either side has a higher incidence of hypercellular, normocellular or hypocellular marrow patterns. A knowledge of the pattern of bone marrow cellularity in various forms of anemia can assist in the interpretation of bone marrow examination and aid the clinical and pathologist in relating the morphologic changes with the underlying disease process. These results can also help make better differentiation of nutritional and non-nutritional causes of anemia and aid in the selection of the appropriate lab tests for confirmation. The present comparative study over one year is therefore carried out to assess the bone marrow cellularity in patients with nutritional and non-nutritional anemia as well as difference in marrow response to the two groups.

Methodology

Study Design: Comparative study was done between patients who presented with nutritional anemia and non-nutritional anemia, and the cellularity of the bone marrow was compared. The study was primarily targeted on the pattern of cellularity in the bone marrow with corresponding morphologic changes in the bone marrow examination.

Study Duration: This study was carried out for one year. All patients who met the following criteria during the study period and who had bone marrow examination as part of their clinical work up were included: - Eligible patients presenting during the study period and who underwent bone marrow examination as part of their clinical evaluation.

Sample Size: 80 patients were enrolled in the study. The subjects of the study were split into two groups. Group I comprised of 40 patients with nutritional anemia and Group II comprised of 40 patients with non-nutritional anemia.

Study Population: Those patients who were diagnosed as anemia by hemoglobin level and other hematological investigations were included. Nutritional anemia was diagnosed when clinical and relevant laboratory examinations indicated iron, vitamin B12 or folate deficiency. Non-nutritional anemia included anemia associated with chronic disease, renal disorders, hemolytic disorders and other known non-nutritional causes.

Inclusion Criteria: Those with evidence of anemia who were clinically indicated for bone marrow aspiration were included. The study was conducted on the patients who had sufficient clinical history, complete blood count, peripheral smear examination, biochemical investigations in relation to the disease. Only those patients for whom adequate data existed for classification of anemia as nutritional or non-nutritional were included.

Exclusion Criteria: Patients with confirmed hematological malignancies, patients with samples of bone marrow aspirate inadequate for diagnosis and patients receiving chemotherapy or radiotherapy were excluded. Patients who lack clinical and laboratory data required for the diagnosis of anemia were also excluded.

Sample Collection: For hematological studies, peripheral venous blood samples were taken with routine aseptic technique. Bone marrow aspiration was done by a trained clinician, under proper clinical conditions and after informed consent. Bone marrow smears were made on the spot and stained by conventional methods.

Hematological Investigations: All patients had a complete blood count (CBC) performed. Laboratory values that were tested were hemoglobin, red blood cell count, total leukocyte count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width. Peripheral blood smear was used to look for microcytic, macrocytic, normocytic, hypochromic or other morphologies.

Biochemical Investigations: Biochemical studies were carried out according to the clinical presentation. Where indicated, serum ferritin, serum iron, total iron-binding capacity, serum vitamin B12 and folate levels were measured. Patients with a possible non-nutritional cause for anemia had renal function tests and other investigations performed. The findings were correlated with clinical and hematological data for classification of patients.

Bone Marrow Examination: The bone marrow aspirate smears were microscopically examined for general marrow cell activity and cellularity. The maturation pattern in erythroid and myeloid series, megakaryocytes and abnormal morphological changes were assessed. Bone marrow cellularity was classified as hypocellular, normocellular or hypercellular, with the age of the patient taken into account and what was considered to be the normal marrow cellularity.

Morphological Assessment: Special emphasis was placed on the erythroid hyperplasia, the megaloblastic changes, erythroid maturation, and myeloid maturation and myeloid to erythroid ratio. The iron stores in the marrow were also evaluated when applicable. Peripheral blood and biochemical investigations were correlated with the morphological findings.

Statistical Analysis: Data were documented on a structured data sheet in the clinical and laboratory setting.

The findings were fed in into appropriate statistics software package for analysis. Numerical data were presented as mean ± SD and categorical data were presented as frequencies and percentages. The distribution of bone marrow cellularity between nutritional and non-nutritional anemia group was done by chi-square test. Statistically significant p-values<0.05 were used.

Ethical Considerations: The study was conducted after obtaining approval from the appropriate institutional ethics committee. Confidentiality of patients' information was ensured during the study.

Informed consent was obtained for bone marrow examination, which was only performed when clinically indicated.

Results

A total of 80 patients with anemia were included in the study. The patients were divided equally into two groups: 40 patients with nutritional anemia and 40 patients with non-nutritional anemia. The results were analyzed based on demographic characteristics, hematological parameters, type of anemia, bone marrow cellularity, and morphological findings.

Distribution of Patients According to Study Group

Table 1: Distribution of study participants

Study Group	Number of Patients	Percentage
Nutritional anemia	40	50.0%
Non-nutritional anemia	40	50.0%
Total	**80**	**100%**

Among the 80 patients, 40 (50%) were diagnosed with nutritional anemia and 40 (50%) with non-nutritional anemia.

Figure 1. Distribution of study participants (N = 80)

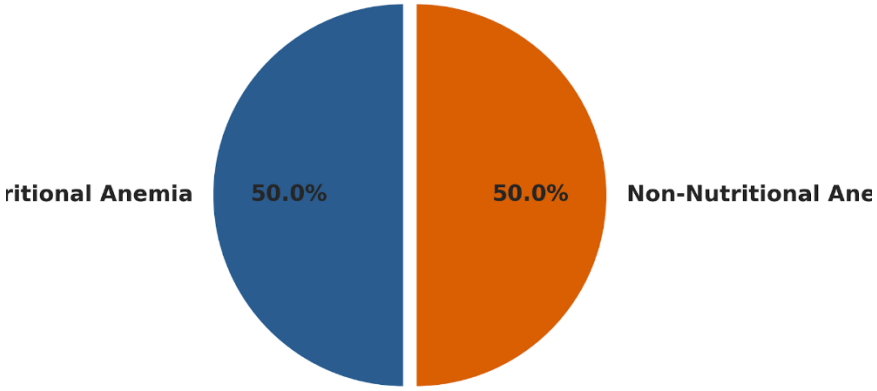


Figure 1:

Age-wise Distribution

Table 2: Age distribution of patients

Age group (years)	Nutritional anemia n (%)	Non-nutritional anemia n (%)	Total
18–30	12 (30.0%)	7 (17.5%)	19
31–40	10 (25.0%)	8 (20.0%)	18
41–50	8 (20.0%)	10 (25.0%)	18
51–60	6 (15.0%)	8 (20.0%)	14
>60	4 (10.0%)	7 (17.5%)	11
Total	**40 (100%)**	**40 (100%)**	**80**

The nutritional anemia group showed a greater proportion of patients in the younger age groups, whereas non-nutritional anemia was relatively more frequent among older patients.

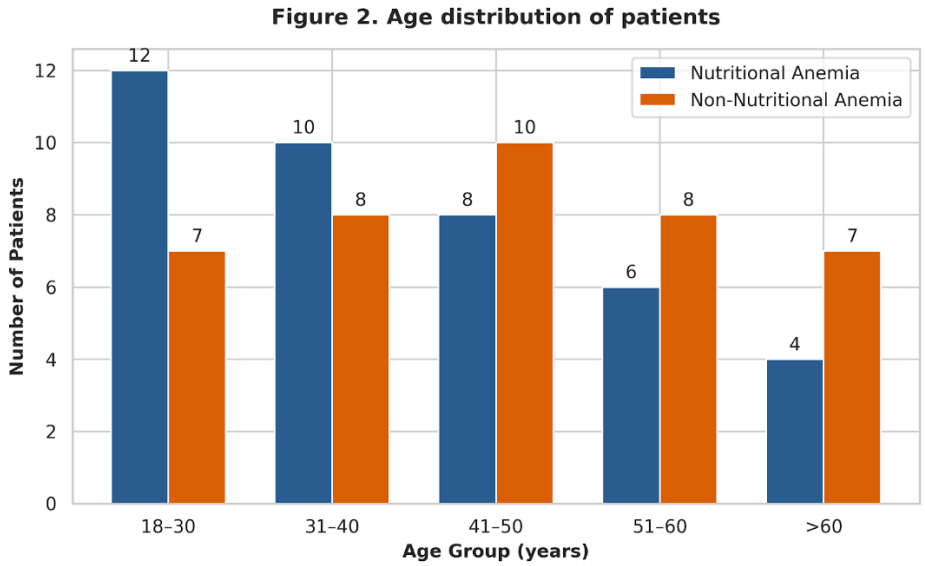


Figure 2:

Distribution of Nutritional Anemia

Table 3: Types of nutritional anemia

Type of nutritional anemia	Number	Percentage
Iron deficiency anemia	22	55.0%
Vitamin B12 deficiency	10	25.0%
Folate deficiency	4	10.0%
Combined nutritional deficiency	4	10.0%
Total	**40**	**100%**

Iron deficiency anemia was the most common nutritional anemia, accounting for **22 (55%)** cases, followed by vitamin B12 deficiency in 10 (25%) cases.

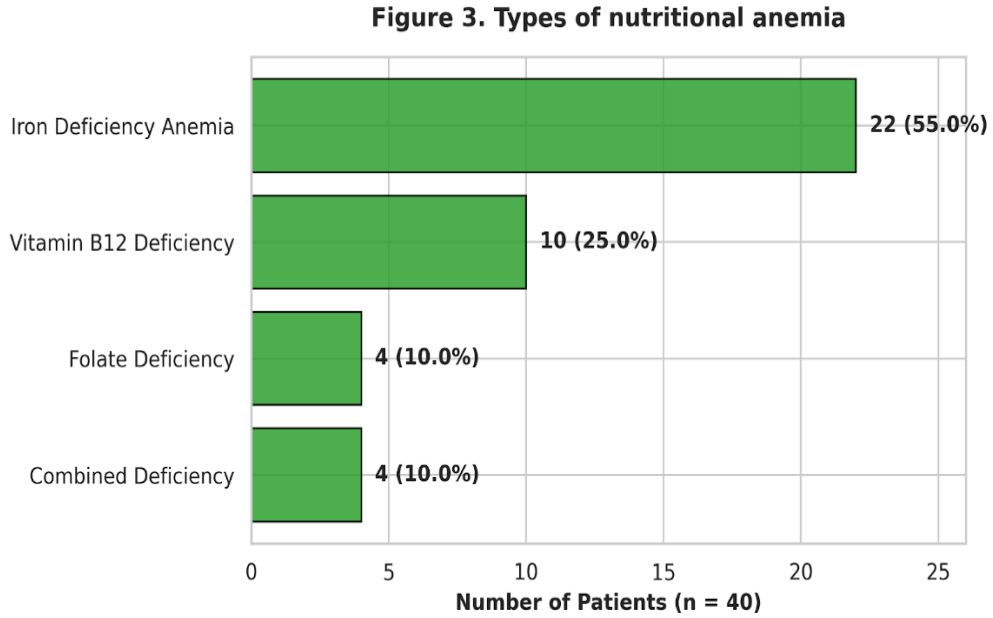


Figure 3:

Hematological Parameters

Table 4: Comparison of hematological parameters

Parameter	Nutritional anemia Mean ± SD	Non-nutritional anemia Mean ± SD
Hemoglobin (g/dL)	7.9 ± 1.4	8.2 ± 1.6
RBC count (million/µL)	3.12 ± 0.61	3.25 ± 0.65
MCV (fL)	78.6 ± 12.4	86.8 ± 9.7
MCH (pg)	25.1 ± 4.8	28.0 ± 3.9
MCHC (g/dL)	31.8 ± 2.1	32.3 ± 1.9
RDW (%)	18.2 ± 3.7	15.4 ± 3.1

The nutritional anemia group showed a lower mean MCV and MCH and a higher RDW compared with the non-nutritional anemia group. This pattern reflects the presence of microcytic and anisocytic changes, particularly among patients with iron deficiency.

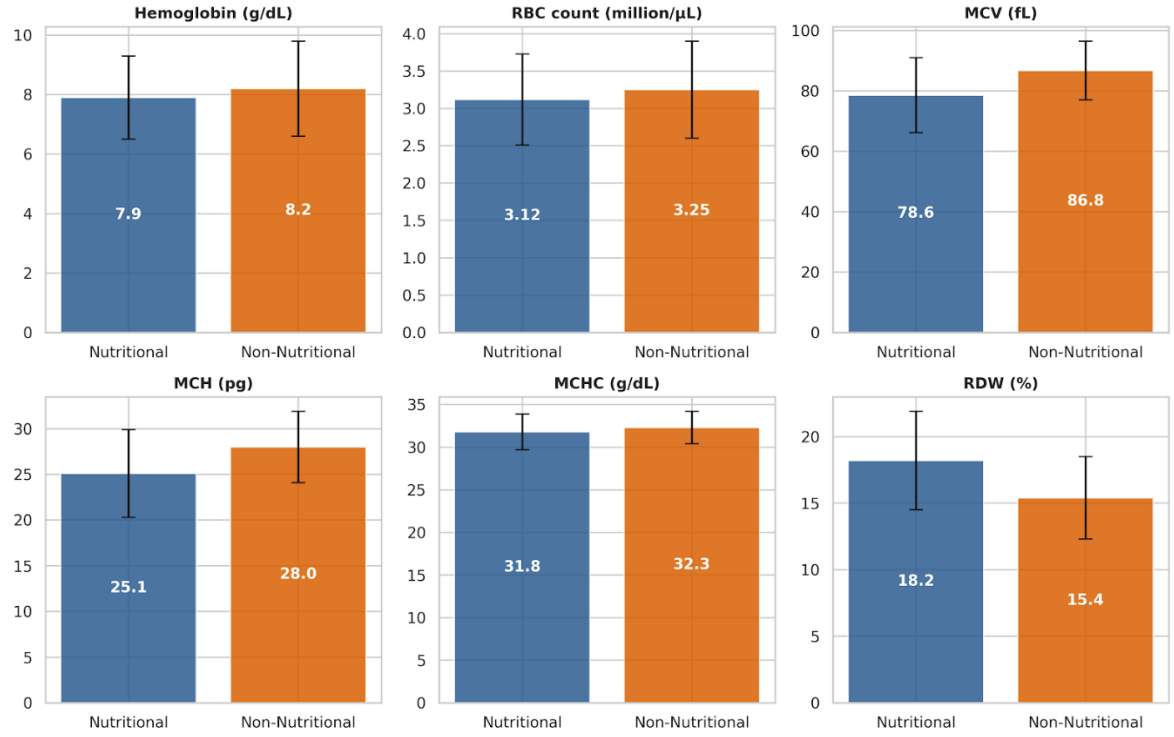


Figure 4:

Peripheral Smear Findings

Table 5: Peripheral smear morphology

Peripheral smear finding	Nutritional anemia n (%)	Non-nutritional anemia n (%)
Microcytic hypochromic	20 (50.0%)	8 (20.0%)
Macrocytic	10 (25.0%)	4 (10.0%)
Normocytic normochromic	7 (17.5%)	22 (55.0%)
Dimorphic	3 (7.5%)	3 (7.5%)
Other	0	3 (7.5%)
Total	**40 (100%)**	**40 (100%)**

Microcytic hypochromic morphology was the most common peripheral smear finding among nutritional anemia patients, while normocytic normochromic morphology predominated in the non-nutritional group.

Figure 5. Peripheral smear morphology

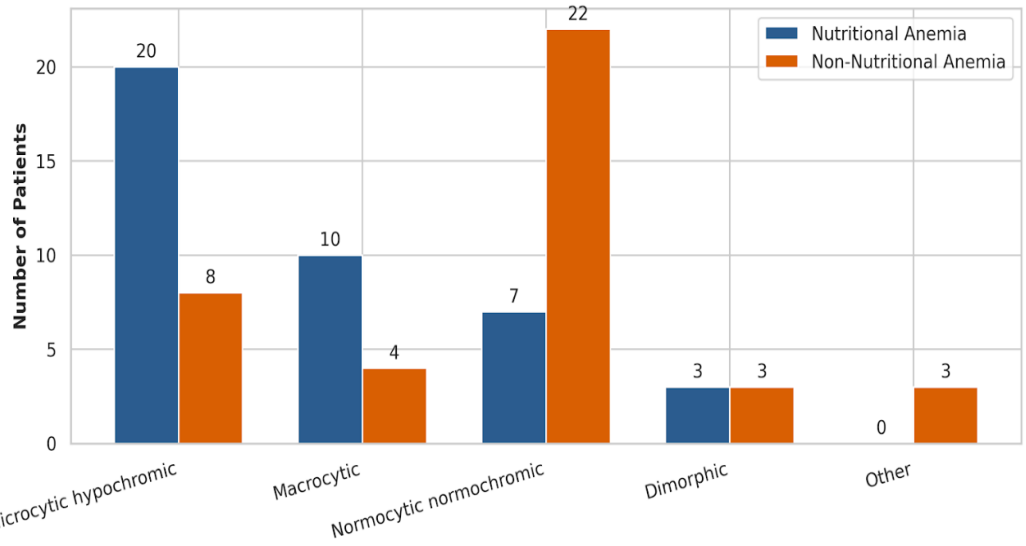


Figure 5:

Bone Marrow Cellularity

Table 6: Comparison of bone marrow cellularity

Bone marrow cellularity	Nutritional anemia n (%)	Non-nutritional anemia n (%)
Hypocellular	3 (7.5%)	10 (25.0%)
Normocellular	11 (27.5%)	18 (45.0%)
Hypercellular	26 (65.0%)	12 (30.0%)
Total	**40 (100%)**	**40 (100%)**

Hypercellular marrow was observed in 26 (65%) patients with nutritional anemia compared with 12 (30%) patients with non-nutritional anemia. Normocellular marrow was more frequent in the non-nutritional group. Hypocellular marrow was observed in 25% of non-nutritional anemia cases compared with 7.5% of nutritional anemia cases.

Figure 6. Comparison of bone marrow cellularity

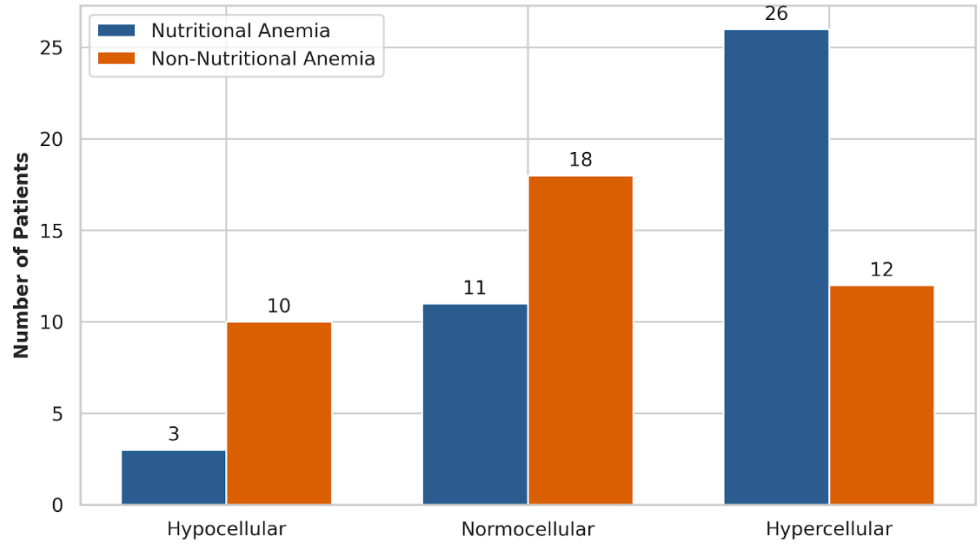


Figure 6:

Bone Marrow Morphological Findings

Table 7: Bone marrow morphological findings

Bone marrow finding	Nutritional anemia n (%)	Non-nutritional anemia n (%)
Erythroid hyperplasia	25 (62.5%)	14 (35.0%)
Megaloblastic changes	12 (30.0%)	2 (5.0%)
Reduced erythropoiesis	5 (12.5%)	12 (30.0%)
Increased M:E ratio	4 (10.0%)	8 (20.0%)
Abnormal megakaryocytes	2 (5.0%)	4 (10.0%)

Erythroid hyperplasia was the most common marrow finding in nutritional anemia. Megaloblastic changes were also considerably more frequent in the nutritional group, particularly among patients with vitamin B12 and folate deficiency.

Figure 7. Bone marrow morphological findings

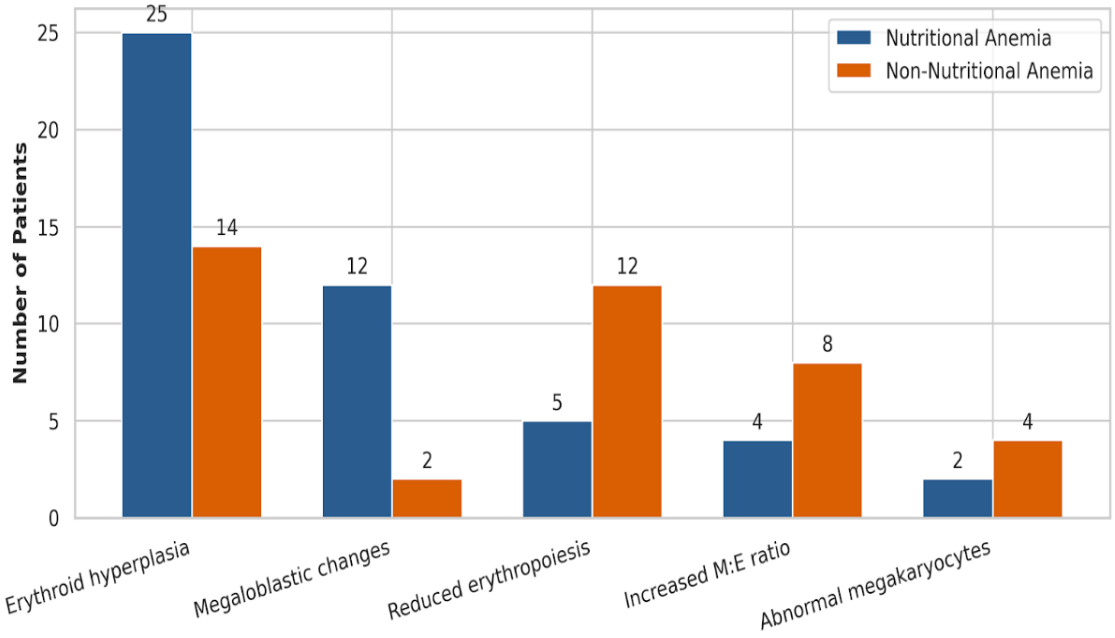


Figure 7:

Comparison of Bone Marrow Cellularity by Nutritional Anemia Type

Table 8: Bone marrow cellularity according to nutritional anemia type

Type	Hypocellular	Normocellular	Hypercellular	Total
Iron deficiency anemia	2	7	13	22
Vitamin B12 deficiency	0	1	9	10
Folate deficiency	0	1	3	4
Combined deficiency	1	2	1	4
Total	**3**	**11**	**26**	**40**

Hypercellular marrow was particularly prominent among patients with vitamin B12 deficiency, where 9 of 10 patients demonstrated hypercellularity.

Figure 8. Bone marrow cellularity according to nutritional anemia type

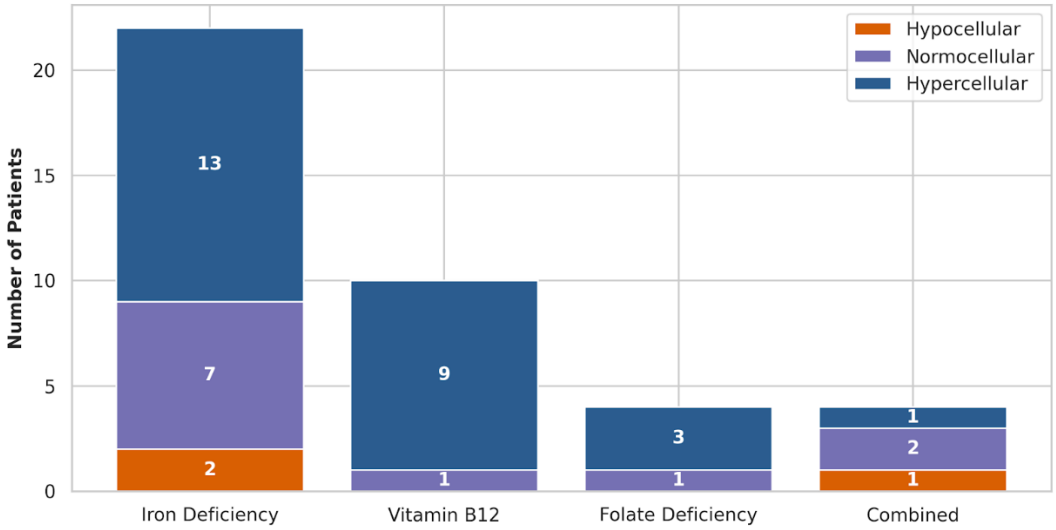


Figure 8:

Comparison of Bone Marrow Cellularity between Groups

Table 9: Statistical comparison of marrow cellularity

Cellularity	**Nutritional anemia**	**Non-nutritional anemia**
Hypocellular	3 (7.5%)	10 (25.0%)
Normocellular	11 (27.5%)	18 (45.0%)
Hypercellular	26 (65.0%)	12 (30.0%)
Chi-square test	**$\chi^2 = 10.15$**	
p-value	**<0.01**	
Significance	**Statistically significant**	

The difference in bone marrow cellularity between nutritional and non-nutritional anemia groups was statistically significant ($p<0.01$). Hypercellular marrow was significantly more frequent among patients with nutritional anemia, while normocellular and hypocellular patterns were more commonly observed in the non-nutritional group.

Discussion

This study aimed to compare the bone marrow cellularity between 80 patients with anemia (group 1, 40 patients of nutritional anemia and group 2, 40 patients of non-nutritional anemia).

The most important finding was that hypercellular bone marrow was more likely to be observed in nutritional anemia while normocellular and hypocellular marrow was more likely to be observed in non-nutritional anemia. Among nutritional anemia, 65% were hypercellular while 30% among non-nutritional anemia was hypercellular.

The nutritional group also had more cases of erythroid hyperplasia. These data indicate that nutritional deficiency (vitamin B12 and folate deficiency) can precipitate excessive, but ineffective hematopoietic activity. However, non-nutritional anemias have several different disease processes; thus, the bone marrow reaction may be quite different.

Previous research: [13] Described the alterations of bone marrow in nutritional deficiencies and emphasised on iron, vitamin B12 and folate as the major nutritional deficiencies that occurs in haematopoiesis. The study also highlighted that there is a possibility of combined deficiency and one form of nutritional anemia can mask the other. This is also applicable in the present study as iron deficiency was the most common nutritional anemia and vitamin B12, folate and combined deficiencies also contributed to the study population. [14] have reported on 168 patients with nutritional anemia in whom they examined the bone marrow iron content. A good correlation was found between the two in uncomplicated IDA; however, there were more discrepancies in the presence of megaloblastic changes. Their study underlines the need to take the bone marrow morphology into account with biochemical investigations and not make judgments based on marrow findings alone. In this study, iron deficiency was the most predominant nutritional subgroup, thus highlighting the need for studies related to iron deficiency in the assessment of

nutritional anemia. [15] studied the erythropoiesis in normal marrow, vitamin B12 deficiency and iron deficiency anemia both morphologically and quantitatively. They were able to show differences in the erythropoietic activity of the various types of nutritional anemia. The present study also revealed hyperactivity of the erythroid cells in nutritional anemia and more frequently in the nutritional group than in non-nutritional group.

Clinical Implications: The results are applicable in the context of testing the patients suffering from unexplained anemia. Bone marrow examination can give further information on the activity and pattern of hematopoiesis: especially when peripheral blood findings and routine biochemical investigations are inconclusive.

Marrow cellularity should be interpreted in conjunction, however, with other laboratory and clinical findings to diagnose nutritional anemia. A hypercellular marrow could be seen in various conditions, so the results should be correlated with the hemoglobin, MCV, peripheral smear, serum ferritin, iron studies, vitamin B12, folate and other appropriate investigations.

Suggestions: The results of this study suggest that patients with anemia should be extensively evaluated to determine the etiology. If there is a suspicion of nutritional deficiency, then appropriate iron, vitamin B12 and folate investigations should follow complete blood count and peripheral smear examination. Bone marrow examination should be performed in cases of unexplained anemia, presence of other cytopenias, and in the presence of abnormal marrow pathology.

A larger number of subjects and preferably subcategories of nutritional group (iron deficiency, Vitamin B12 deficiency, folate deficiency and combination) should be included in future studies. Multicentre studies can also lead to better evidence and can enhance the generalizability.

Limitations: This study comprised relatively small numbers of patients (80) and a 1-year period. In the non-nutritional anemia group, there were various underlying diseases that could have been a cause of the variation of marrow cellularity.

Routine microscopic examination of cellularity also may be subject to some observer variation. Thus, more extensive investigations that rely on a standardized and/or digital means to assess cellularity is recommended.

Conclusion

In the present study, the cellularity of bone marrow was compared between the nutritional and non-nutritional anemia patients after one year. A total of 80 patients were included in which 40 patients were from each group. The results showed that bone marrow cellulosity, in both groups, was significantly different.

Nutritional anemia was associated with hypercellular marrow more often than other types of anemia, especially those caused by folate and vitamin B12 deficiencies. Nutritional anemia group was also more likely to have erythroid hyperplasia and megaloblastic changes. However, there was a higher percentage of normocellular and hypocellular marrow in non-nutritional anemia patients. The study suggests that bone marrow cellularity can supplement with valuable information on underlying mechanism of anemia. Cellularity alone, however, is not sufficient to determine the etiology and is to be correlated with the complete blood count, peripheral smear, iron profile, vitamin B12, folate, renal function and other pertinent tests. In general, bone marrow studies, if clinically indicated, can be very helpful in differentiating and assessing (nutritional and non-nutritional) anemia. These findings should be confirmed by further studies on larger numbers of people using standardized methods of looking at the cellularity.

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