Abstract

Background: More than 1.5 billion people are suffering with anaemia worldwide. Various changes in morphology of RBC have been found during anaemia. An insignificant high count of total leucocyte count (TLC) was found in anaemic compare to healthy individual. Whereas, neutrophils and basophils were high in anaemic patients. Therefore, the present study was designed to assess the prevalence of anaemia and its relation with morphology of RBC and WBC in elderly population. Subjects and Methods: The present study included 168 elderly anaemic patients. IDA (36 patients) was the most common type of anaemia. Results: Normocytic normochromic blood picture was found in 72 (42.8%) patients and these included cases of anaemia of chronic disease, multiple myeloma, carcinomatous marrow deposits and anaemia of renal disease. Macrocytic predominant blood picture along with poikilocytosis in the form of ovalocytes were seen in smears of 36 (21.4%) patients of megaloblastic anaemia, myelodysplastic syndrome and myelofibrosis. Microcytic hypochromic predominance was seen in all the 36 (21.4%) patients of iron deficiency anaemia. Conclusion: Nutritional anaemia was the most commonly prevalent anaemia among the elderly population. Predominantly microcytic hypochromic RBCs, pencil cells and a few target cells with increased platelets are indicative of iron deficiency anaemia. These patients also had low MCV values. Bone marrow aspiration smears revealed low iron stores. Anaemia is considered the disease with multifactorial etiology, however, we hope that morphology of RBC and WBC along with other haematological findings might be helpful in finding the possible causes of anaemia.

Keywords: Elderly, Anaemia, Morphology, IDA.

Introduction

Decrease in number of RBC or oxygen carrying capacity of blood is considered as anaemia which may vary due to different factors like age, sex, environment etc. More than 1.5 billion people are suffering with anaemia worldwide.[1] Haemoglobin is an integral part of RBC; which are produced by bone marrow with the help of several nutrients and erythropoietic factor.[2] Various changes in morphology of RBC have been found during anaemia. Decrease of vitamin B2 and B6 can lead to microcytic whereas, decline of B12 induces synthesis of macrocytic RBC.[3] Anaemia has been found associated with ischemia and hypoxia. An insignificant high count of total leucocyte count (TLC) was found in anaemic compare to healthy individual. Whereas, neutrophils and basophils were high in anaemic patients.[4] Differential diagnosis of anaemia is still difficult for smaller health centres with limited facilities. Moreover, sometimes it is hard to differentiate anaemia of chronic disease with iron deficiency anaemia.[5] Type of anaemia and differential diagnosis of it can be obtained by morphologic assessment of RBC and WBC along with various other haematological findings.[6] Mean cell volume (MCV) is also an important parameter for the diagnosis as well as classification of anaemia.[7]

Therefore, the present study was designed to assess the prevalence of anaemia and its relation with morphology of RBC and WBC in elderly population.

Subjects and Methods

Study design

In a Tertiary Care Hospital a total of 168 geriatric patients of age 65 years and above, male and female patients with geriatric level anaemia (Hb < 12 g/dl) admitted, were included in the study. Among these patients 92 were male and 76 were female patients. Informed and written consent from these patients was taken after explaining them the importance of study. A thorough physical and systemic examination, clinical history, family history, drug history and history of chronic diseases were documented before going for detailed relevant investigations. The haematological investigations with supporting biochemical parameters were estimated, which included complete blood count, serum ferritin test, peripheral blood
film examination, iron studies, serum B12 and folic acid levels, bone marrow cytology, liver and renal function tests, urine examination and radiological examination, when required. Complete blood count included the following: Hb level; total erythrocyte count; red cell indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC); and white cell indices such as total leucocyte count (TLC) and differential leucocyte count, as well as platelet count, packed cell volume, reticulocyte count and corrected TLC. All investigations were carried out on an automated cell counter (XS-800i63591Analyse APL00-13; Sysmex Corporation, Kobe, Japan).[6] The erythrocyte sedimentation rate (ESR) was determined using Westergren's method and the differential leucocyte count in the case of morbid conditions like leukaemia was confirmed using Leishman’s stain and subsequently studied under a microscope using an oil immersion lens.[7] Serum ferritin test
Bone marrow examination
Bone marrow aspiration, trephine biopsy, marrow section and staining of slides were carried out following standard protocol.[8]

**Iron studies**
Iron studies included serum iron, total iron binding capacity (TIBC) and serum ferritin levels. Plasma iron saturation (%) were calculated using the following standard formula:

\[
\text{Serum iron} \times 100 \\
\text{TIBC}
\]

1. Serum vitamin B12 and folic acid levels were estimated by means of the Cobase-411 (HITACHI) automated analyser.
2. Urine examination, including routine, microscopic and examination for Bence Jones proteins, was also carried out to investigate for multiple myeloma.[9]
3. Liver function tests and renal function tests were performed using Siemens Dimension AR automated analyser.

**Radiological investigations**
Radiography of the chest, flat bones and skull, ultrasonography of abdomen, computed tomographic scan and MRI were carried out when required.

**Other investigations**
The patients were investigated thoroughly to diagnose the underlying disease. This included endoscopy of the upper GIT, mammography, prostatic specific antigen estimation and serum rheumatoid factor.

**Results**
The present study included 168 elderly anaemic patients. [Table 1] shows that IDA (36 patients) was the most common type of anaemia followed by anaemia of chronic diseases (34 patients), dual deficiency anaemia (24 patients), megaloblastic anaemia (20 patients) and marrow infiltration anaemia (14 patients). Rest of the prevalent types of anaemia were multiple myeloma, myelodysplastic syndrome and myelofibrosis.

![Figure 1: Prevalence of different types of anaemia.](image)

**Peripheral blood film (PBF)**
Detailed PBF: Examination was done in 168 patients of anaemia, which included the RBC morphology, WBC morphology and morphological details of platelets. Rough estimation of total leucocyte count and platelet count was done.

<table>
<thead>
<tr>
<th>Table 1: RBC morphology in elderly patients.</th>
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<tbody>
<tr>
<td><strong>RBC morphology</strong></td>
</tr>
<tr>
<td>Normocytic normochromic predominance</td>
</tr>
<tr>
<td>Microcytic hypochromic predominance</td>
</tr>
<tr>
<td>Macrocytic predominance</td>
</tr>
<tr>
<td>Dimorphic blood picture</td>
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<tr>
<td>Ovalocytes</td>
</tr>
<tr>
<td>Pencil cell (elliptical forms)</td>
</tr>
<tr>
<td>Target cells</td>
</tr>
<tr>
<td>Teardrop cells</td>
</tr>
<tr>
<td>Polychromasia</td>
</tr>
<tr>
<td>Cabot ring</td>
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<tr>
<td>Basophilic stippling</td>
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<tr>
<td>Rouleaux formation</td>
</tr>
<tr>
<td>nRBCs/100 WBCs</td>
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</tbody>
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<tr>
<th>Table 2: Estimation of total leucocyte count.</th>
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<tr>
<td><strong>Total count</strong></td>
</tr>
<tr>
<td>Normal</td>
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<tr>
<td>Decreased</td>
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<tr>
<td>Increased</td>
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<tr>
<td>Total</td>
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**RBC morphology**: The peripheral blood films were examined in detail for RBC morphology, including size and shape of RBCs, chromia, presence of inclusions, evidence of rouleaux formation, number of nucleated RBCs/100WBCs and presence or absence of features of dyscrhypoisis. Normocytic
normochromic blood picture was found in 72 (42.8%) patients and these included cases of anaemia of chronic disease, multiple myeloma, carcinomatous marrow deposits and anaemia of renal disease. Macrocytic predominant blood picture along with poikilocytosis in the form of ovalocytes were seen in smears of 36 (21.4%) patients of megaloblastic anaemia, myelodysplastic syndrome and myelofibrosis. Microcytic hypochromic predominance was seen in all the 36 (21.4%) patients of iron deficiency anaemia. Dimorphic blood picture was seen in 24 (14.3%) patients of dual deficiency anaemia. Rouleaux formation was seen in patients of anaemia of chronic disease and multiple myeloma. Tear drop cells were seen in patients of myelofibrosis and myelophthisic anaemia. Dyserythropoietic features were seen in cases of megaloblastic anaemia, myelodysplastic syndrome and myelofibrosis.

As shown in [Table 2] peripheral blood smears were screened for total leukocyte counts, and they were found increased in 26.2% patients, decreased in 16.7% and normal in 57.1% patients.

![Figure 2: WBC morphology in elderly patients](image)

Morphological details of white blood cells, differential leukocyte count and presence of any immature cells were also looked for. As shown in [Figure 2] blasts were seen in smears of patients with acute leukemia, 4 of the 8 cases of myelodysplastic syndrome and myelofibrosis. Promyelocytes were seen in 12 patients and myelocytes in 36 cases. Toxic changes in the form of cytoplasmic vacuolations, toxic granules and döhle bodies were seen in patients of infection and septicemia. Circulating plasma cells were present in the smears of 8 patients of multiple myeloma. Absolute lymphocytosis and many smudge cells were seen in 2 patient of chronic lymphocytic leukemia.

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>No. of patients</th>
<th>Percentage (%)</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51</td>
<td>60.9%</td>
</tr>
<tr>
<td>Decreased</td>
<td>16</td>
<td>19.0%</td>
</tr>
<tr>
<td>Increased</td>
<td>17</td>
<td>20.2%</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>100%</td>
</tr>
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As shown in [Table 3] peripheral blood smears were also screened for platelet adequacy. Platelet count was estimated and morphological details were looked for. Giant platelets were seen in the patients of myelofibrosis.

Discussion

In our study haemoglobin values were variable depending upon the various causes of anaemia. Anaemia was divided into mild (Hb 9-12 gm/dl), moderate (Hb 6-9 gm/dl) and severe (Hb<6gm/dl) degree. Patients with nutritional deficiency anaemia, MDS and myelofibrosis, had moderate to severe degree of anaemia. Almost all the patients of carcinomatous marrow deposits, multiple myeloma and acute leukaemia had severe degree of anaemia while patients of anaemia of chronic disease had mild to moderate degree of anaemia.

A growing body of medical literature supports the contention that mild anaemia or a low haemoglobin level is associated with broad range of poorer health-related outcomes, both in specific disease entities and all cause mortality for general population e.g., patients with heart failure whose haemoglobin levels are in the lowest quartile have more symptoms, poorer haemodynamics, and greater mortality than those with higher haemoglobin levels, and these differences are particularly marked in the elderly.[10]

Findings of the present study showed that nutritional deficiency anaemia was the most common type of anaemia in our study population. Further, ACD, marrow infiltration and multiple myeloma were the other major type of anaemia which were prevalent among elderly population. In addition, few cases of AML, CML and anaemia due to renal diseases were found.

These findings are in agreement with the earlier studies of Milman N et al.[11] as they observed IDA was the most common type of anaemia found in elderly healthy Danish population. Similarly, Coenen JL et al.[12] recorded iron deficiency was the predominant cause of anaemia in their study population. In addition, Ezekowitz JA et al.[13] conducted a study on 12,065 patients with median age of 78 years. Among these subjects 17% of the patients had anaemia. Iron deficiency was present in 21% of cases, 58% had anaemia of chronic disease while others suffered from miscellaneous conditions.

Haemoglobin and packed cell volume were uniformly low whereas results of red cell indices i.e. MCV, MCH and MCHC were variable depending upon the underlying cause of anaemia. MCV was within the normal range in almost all cases of anaemia of chronic diseases (ACD) and dual deficiency anaemia. MCV value was less than 70 fl in cases of iron deficiency anaemia while MCV values were more than 110 fl in patients of megaloblastic anaemia, myelodysplastic syndrome and myelofibrosis.

These results are very similar to the findings of the earlier study of Means RT et al.[14] as they recorded MCV is almost normal in ACD with only an occasional patient having low MCV value. Values below 72 fl are rare in ACD and more in favour of iron deficiency anaemia. Similarly, Guyatt GH et al.[15] described in their study that MCV values less than 74 fl made the diagnosis of iron deficiency anaemia more
likely whereas MCV value of more than 95 fl made the diagnosis of iron deficiency anaemia less likely. Alike, Mahmoud MY et al.,[16] in their study assessed the causes of macrocytosis with MCV>95 fl in 124 patients of age 75 years and above. They found that an elevated MCV value was a useful parameter directing further investigations, which ultimately yielded a definite diagnosis in 81 patients out of 124 in their study. At higher MCV value more than 100 fl, most of their patients were diagnosed as having megaloblastic anaemia, megaloblastic syndrome or chronic liver disease. Macrocytosis in rest of the cases could not be explained even after exhaustive investigations. They hypothesised that these cases could represent MDS in evolution. In their study reticulocyte count was initially low in the patients of severe megaloblastic anaemia but rapid elevation in the reticulocyte count was observed after vitamin B12 and folic acid therapy. Reticulocyte count was also observed to be higher in patients having active bleeding. Detailed peripheral blood smears were studied in all the patients and we found them to be of considerable diagnostic importance in evaluating anaemia in the elderly. Brill JR et al.,[17] mentioned in their study the importance of detailed peripheral blood smear examination in the evaluation of anaemia in the elderly. The detailed examination of peripheral blood smears yielded important diagnostic clues and confirmatory evidence. Vu TTM et al.,[18] also stressed the importance of peripheral blood smear examination in initial investigation of anaemia in elderly. Smieja MG et al.,[19] also documented the importance of peripheral blood smear examination in recognizing and investigating anaemia in the hospitalized elderly people. Various studies suggest that MCV (microcytic, macrocytic and normocytic) along with RDW might be very helpful for the clinician in the diagnosis of the types of anaemia. Decreased TLC along with altered morphology of WBC and RBC have been found helpful in the differential diagnosis of anaemia. Variations in TLC were recorded in the present study. However, normal count was more common in comparison to high or low TLC. Moreover, morphological changes in WBC were more common for leukemia, multiple myeloma and diseases conditions which are well known to interfere with morphology of WBC. 

Several studies have shown decreased physical performance and strength in elderly anaemic patients. Pennix BW et al.,[21] found that decrement in performance of three different times functional tests Standing balance, five repetitions of sitting and rising from a chair and an 8 foot walk) roughly correlated with declining haemoglobin concentrations in community-dwelling elderly men and women. Therefore timely and exact diagnosis of anaemia is essential for elderly population.

Conclusion

Nutritional anaemia was the most commonly prevalent anaemia among the elderly population. However, various other types of anaemia due to different pathologies may also be found in elderly population. Therefore, exact and early diagnosis of anaemia might be of prime importance for clinician for the treatment of anaemia in elderly subjects. In addition, predominantly microcytic hypochromic RBCs, pencil cells and a few target cells with increased platelets are indicative of iron deficiency anaemia. These patients also had low MCV values. Bone marrow aspiration smears revealed low iron stores. Anaemia is considered the disease with multifactorial etiology, however, we hope that morphology of RBC and WBC along with other haematological findings might be helpful in finding the possible causes of anaemia.

References

Raina & Gupta; Various Types of Anaemia and Their Relation with Morphology of RBC and WBC


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