**MANUSCRIPT (MAIN FILE)**

TITLE: Clinicopathological profile of anaemia cases in adult (20-60 years) attending a Rural HospitaL

**ABSTRCT**

**Background: :** Anemia is a worldwide problem with highest incidence in developing countries. India has the highest prevalence of nutritional anemia predominantly in women and children.

**Methods:** An observational and analytical study was carried out for period of 12 months. We studied 261 adult patient for typing the anemia. We perform the hematology parameter by automated hematology analyzer, For confirmation PBS and bone marrow examination, serological test like serum iron profile , vit B12 and folic acid levels in blood is done

**Result:** Total 261 cases of anemia included in the study. Microcytic hypochromic anemia were 63.2 %of cases, macrocytic anemia cases were 36.77% of cases. Large number of iron deficiency anemia’s were seen in female with reproductive age group and megaloblastic anemia seen with age 50-60 years of age in both sex.

**Conclusion:** For the diagnosis of nutritional anemia haemogram by automated hematology parameter, PBS, serum iron profile , serum vit B12 and folic acid is required

**KEYWORDS:**

 Iron deficiency anemia, megaloblastic anemia, Nonmegaloblastic macrocytic anemia, Nutritional anemia.

**MAIN TEXT**

**INTRODUCTION**:

In 1992, World Health Organization (WHO) global estimates of anemia prevalence averaged 56%, with range of 35-75% depending on geographic location. Prevalence of anemia seen in south Asia, among highest in world. In India recent data from national family health survey 1998/1999 stated that woman with reproductive age have higher prevalence rate concentration and impaired capacity to transport oxygen.[1]  It has multiple factor such as genetic- haemoglobinopathies, infectious- malaria, intestinal helminthes and nutritional- which includes iron deficiency as well as deficiency of vitamins such as folate vitamin A, B12 and minerals like cupper. [2]

 The evaluation of cause of anemia includes complete blood count, peripheral smear, and reticulocyte count and serum iron indices. [3] As per WHO classification, majority of subject 41.3% suffered from moderate anemia, while 18.4and 0.4 suffer from mild and severe anemia. In the study of Neelam Deshpande et al 70% of anemic subject had low MCV with high RDW suggestive of iron deficiency. [4]-

 Uma Khandri and Archana Sharma stated that megaloblastic anemia was diagnosed from complete blood count, red cell indices, blood film examination and assay of two vitamins. Marrow examination was not essential for diagnosis. Cobalamine deficiency was responsible for megaloblastic anemia in majority of patient (65%), combined ( folate and cobalamine) seen in 12% and pure folate deficiency in 6% cases. [5]

Hence the present study was carried out to find out commonest type of anemia in the rural population.

**MATERIALS AND METHODS:**

This is prospective study comprised of adult from outpatient and indoor department of a tertiary care in teaching hospital in Maharashtra, India. The period of study was from January 2015 to December 2015. Total 261 patients with anemia’s were selected for study with informed consent.

**\*Selection Criteria for cases;**

Inclusion criteria (cases included in study)

* Male and female patient in the age group 20 to 60 years.
* Patient with HB value 10gm/dl or less.

Exclusion criteria ;( cases excluded from study)

* Pregnant and lactating women due to physiological anemia
* Patient who were on treatment / therapy for any reason

Haemogram were performed by automated hematology analyzer sysmex XN 1000. This instrument performed hematology analysis according to hydrodynamic focusing, flow cytometry method and SLS hemoglobin method.

 Microcytic hypochromic anemia and macrocytic anemia were included in the study.

The haemogram provided the following parameter;

1. Hb (Hemoglobin)
2. Red Blood Cells (RBCs) count
3. Hematocrit (PCV)
4. Mean Corpuscular Volume (MCV)
5. Mean Corpuscular Hemoglobin (MCH)
6. Mean Corpuscular Hemoglobin Concentration(MCHC)
7. Red cell Distribution Width (RDW)

Cases are classified in to microcytic and macrocytic anemia on the basis of MCV. Microcytic anemia is identified when MCV is <80fl, macrocytic anemia is identified when MCV exceeds 100fl, increased RDW in both and confirmed by Peripheral Blood Smear (PBS). PBS examinations were performed, we observed for anisopikilocytes, microcytes and macrocytes. Variation in size and shape called as anisopoikilocytosis. Microcyte is evaluated by comparison with diameter of small lymphocyte nucleus which is approximately the same size of of normal RBC.[6]

 Additional 5ml blood sample collected for special investigation for S. ferritin, S iron, TIBC, % saturation for confirmation of microcytic anemia and S vit B12 and S folic acid estimation in macrocytic anemia.

 Normal Serum (S) value of Ferritin, iron, Total Iron Binding Capacity(TIBC) , %saturation, vit B12and folic acid:

1. Ferritin; Male =20-70ng/ml, Female=6-40ng/ml
2. Iron; Male=27-138ug/dl. Female=33-102ug/ml
3. TIBC; Male =174-31ug/ml.. Female =194-372ug/ml
4. % Saturation; Male =0.06-0.33. Female =0.06-0.33
5. Vit. B12; Male=221-911pg/ml, Female =221-911pg/ml
6. Folic acid; Male= >5.38ng/ml, Female = >5.38pg/ml

Values below the normal range is consider as decreased and values above normal range consider as increased.

Methods for Serum ferritin, iron, TIBC, % transferrin saturation , vit B12 and folic acid:

1. S. ferritin, vit B12 and folic acid; Fully automated Bidirectionally Interfaced Chemi Luminescent Immuno Assay.
2. S iron; Ferrozine method without deproteinization
3. Spectrophotometric assay
4. Transferrin saturation; Derived from iron and TIBC values.
5. Vit B12 and folic acid; Fully automated Bidirectionallym Interfaced Chemi Luminescent Immuno Assay.
6. Folic acid; Fully automated chemiluminicent assay.

Values done by automated hematology analyzer correlated with PBS findings, S. iron profile value, Vit B12 and folic acid levels.

**Criteria for diagnosis of various anemia (6)**

**1 Microcytic hypochromic anemia**

1. Iron deficiency anemia: S. Ferritin; ↓ed (decreased)

 S. Iron; ↓ed

 TIBC; ↑ed (increased)

 % Saturation; N/↓ed

 Bone marrow aspiration; Erythroid hyperplasia and

 Micronormoblastic maturation

 Prussian blue stain: Grade- zero to one

1. Anemia of chronic diseases:

 S. Ferritin; ↑ed

 S. Iron; ↓ed

 TIBC; Normal/ ↓ed

 % Saturation; Normal

 Bone marrow aspiration; Erythroid hyperplasia and

 Micronormoblastic maturation

 Prussian blue stain; Grade- Two to five

 c) Hemoglobin deficiency syndrome:

 S. Ferritin; Normal

 S. Iron; Normal

 TIBC; Normal

 % Saturation; Normal

 Bone marrow aspiration; marked erythroid hyperplasia

 Prussian blue stain; Grade two to six

In cases of Hb deficiency syndrome cases with normal RDW and increased reticulocyte count, reticulocyte count was performed manually using supravital stain with methylene blue- showed dark blue granules in the cell identified as reticulocyte.

 Advised them to perform hemoglobin electrophoresis.

**2 Megaloblastic anaemia**:

1. PBS showed hyper segmented neutrophil –At least one six lobed neutrophil of 100 cells examined. Also examined macroovalocytes.
2. Bone marrow examination - Diagnostic criteria; Presence of Polychromatic megaloblast.
3. Decreased Serum Vit B12 and folic acid levels below the normal level.

**3 Non megaloblastic macrocytic anaemia**:

Bone marrow shows macrocytic ertyhroid hyperplasia, no hypersegmented neutrophil or macroovalocyte, S B12 assay between 254-683 ug/ml and folic acid normal / decreased we performed reticulocyte count, few cases showed increased in reticulocyte count, these cases are advised to investigate for hemolytic anemia

**RESULT**

 Total number of cases in study: 261

**Table 1 : Age and sex distribution of anaemia in study;**

 **Graph 1) Distribution of cases of anemia in the study: Total= 261**

**Graph 2) Distribution of cases according to type of anemia:**

 **Graph 3)Distribution of cases of Microcytic hypochromic anaemia: Total; 165**

**Table 2 Distribution of cases according to age groups in microcytic hypochromic anemia:**

Large number of iron deficiency anemia seen in female with age group of 20-29 years, followed by age group of 30-39 years, in reproductive age group there is no specific group affected in other type of anemia’s.

**Graph 4) Distribution of cases with Macrocytic anaemia:Total;96**

**Table3**

 **Distribution of cases according to age group in macrocytic anemia**

In nonmegaloblastic macrocytic anemia, cases with increased reticulocyte were ask to investigate for hemolytic anemia.

**Table 4**

 **Clinical features of microcytic hypochromic anemia:**

**Table 5**

**Clinical features -Macrocytic anemia:**

**DISCUSSION:**

In women of childbearing age, the anemia prevalence is 30.2%. Over all 468.4 million women of childbearing age are anemic. The highest prevalence is found in Africa (47.5%) and in South-East Asia (35.7%). It is 17.8% in America, 14% in the United Arab Emirates; and from a low of 11% in Egypt to over 40% in the Syrian Arab Republic. Iron deficiency anemia was considered as important contribution of anemia in global burden of anemia in WHO report 2002. [7]

National Family Health survey in 2005-2006 showed, the prevalence of anemia was 55% in female aged 15-49 years and 24% in male aged15-49 years. According to World Health Organization, there are two billion people with anemia in the world and half of anemia due to iron deficiency. [8]

In developing countries prevalence of nutritional anemia is 40%, among the various nutritional anemia iron deficiency anemia is most common It is the most common nutritional disorder worldwide and accounts for approximately one-half of anemia cases. [4, 9] In our study male was 36.78% and female was 63.21%. Iron deficiency anemia was 40.22%, more commonly seen in female with reproductive age group.

 Patient with iron deficiency anaemia has history of decreased work or exercise tolerance, shortness of breath, palpitation. (6) In the etiologies for iron deficiency anemia Terri D et al and Mathew W Short et al stated that it include blood loss like menorrhagia, epistaxis, melena, hematuria, and hematemesis. In developing countries decreased intake is primary cause of iron deficiency anemia.. Other causes of anemia include chronic blood loss from gastrointestinal tract, gynecological disorder and genitourinary blood loss. Gastrointestinal bleeding can be acute or chronic. Patient present with maroon coloured stool or blood in stool. Bleeding may be associated with NSAID or aspirin In gynecological disorder postmenopausal women with excessive menstruation seen. [3,9]

In present study in microcytic hypochromic anemia patient present with symptoms like fatigue, palpitation, menstrual bleeding, few of them present with joint pain, Gastrointestinal bleeding, dyspnoea on exertion which is similler to finding of Terri D et al [3] and Mathew W Short et al[9]

According to Mathew W Short et al diagnosis of iron deficiency anemia requires laboratory confirmed evidence of anemia as well as evidence of low iron stores. Values consistent with iron deficiency include a low serum iron level, low transferrin saturation and high total iron binding capacity. Serum ferritin is particularly valuable in anemic patient because level below 12ug/L is diagnostic of iron deficiency anemia [9,10,11]

In our study, 40.22% cases were with iron deficiency anaemia, 36.01% are female and 04.21% are male, showed a decreased parameter below the normal range like serum iron, ferritin, transferrin saturation and increased TIBC above the normal level, similar finding with Mathew W Short et al.[9]

Other causes of microcytosis include chronic inflammatory state, lead poisoning and thalassemia and sideroblastic anemia. [9] Hemoglobin level less than 9 gm/dl in patient with microcytic anemia and normal iron studies suggest Hb H disease, B thalassemia major or intermedia. An increased RDW may be particularly helpful in distinguish between iron deficiency and thalassemia minor. A bone marrow aspirate for iron stain remains gold standard for iron deficiency anemia in difficult cases. [12 ] We performed Prussian blue stain for iron store in cases wherever it is possible in Iron deficiency anemia and showed decreased grading.

Ferritin is acute phage reactant protein, serum levels tend to be elevated in inflammatory condition.[12,13,14,15]

Ferritin is elevated in inflammation, autoimmune disorder, chronic infection and liver disease. Elevated levels of ferritin well established in still’s disease, multiple sclerosis and rheumatoid arthritis. Ferritin also plays an important role in host immune response is evident from its increased concentration.[13] In anemia of chronic inflammation ferritin levels rises moderately achieving mean level such as 300-400ug/L Ferritin criteria used for recognition of coexisting iron deficiency anemia in patient who have chronic inflammation .In the study of Sheetal Patel et al observed massive hyperferritinemia in adult onset stills desease.[14]

In our study 14.93% of cases showed increased ferritin above 100ng/ml, 8.42% are female and 6.51% are male. Cases include various inflammatory conditions like respiratory disease, chronic urinary disease, rheumatoid arthritis and 2 cases of heart diseases

Megaloblastic anemia is more common in vegetarian than nonvegetarian. Folic acid deficiency more commonly seen in elder, children, pregnant women and haemolytic anaemia. Clinical feature of megaloblastic anaemia are anorexia, irritability, fatigue, palpitation. Commonest physical finding was pallor, fever, generalized weakness, splenomegaly, hepatomegaly and hypopigmentation. Common neurological manifestation of vit B12 deficiency includes parasthesia, weakness, gait abnormalities and behavior changes. [16,17,18]

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In the present study most of patient with macrocytic anemia presented with lethargy, tingling numbness, loss of appetite, loss of weight, pain in abdomen, fever and alcoholism

For laboratory diagnosis of megaloblastic anemia complete blood picture with PBS showed macrocytosis, hypersegmented neutrophil, raised MCV and RDW, assay of two vitamins (vit B12 and folic acid) and bone marrow aspiration required for definitive diagnosis.[16,17,18]

The severity of megaloblastic marocytosis is directly proportional to the severity of the anaemia and early megaloblastosis may manifest only mild change. Six lobed neutrophil may be absent and increased in four lobed and five lobed neutrophil may be evident or presence of one seven loabed neutrophil or two six lobed neutrophil or three five lobed neutrophil strongly suggest megaloblastic anemia. [12]

Anisocytosis and poikilocytosis is higher in megaloblastic anemia. [16,19]

We observed anisopoikilocytosis in most of cases in present study.

In our study 27%cases showed megaloblastic anemia On PBS showed macrocytes, macrovalocyte, occasional or one-two hypersegmented neutrophil or hypersegmented neutrophil not be seen in few cases, increased MCV, between 110-131fl, on haematology automated analyzer and presence of megaloblast in bone marrow aspiration.

 The finding of MCV of more than 100cu microns quite helpful due to limited differential diagnosis. An MCV of more than 120cu microns almost always indicate megaloblastic anemia. If there is any doubt as to cause of macrocytic anemia, a bone marrow aspirate and biopsy should be done, in classic megaloblastic anemia one will see increased cellularity in the biopsy specimen. The aspirate will show erythroid hyperplasia, delayed nuclear maturation relative to haemoglobinization of the cytoplasm seen best in basophilic, polychrmatophilic and orthochromatophilic erythroblast.[12]One author stated only minority of patient with MCV level above 100fl are deficient in vitB12 or folate. [,20]

Moreover serum cobalamine levels may not be indicative of actual deficiency. Cobalamine levels may be falsely high in patient with megaloblastosis due to nitrous oxide, transcobalamine II deficiency.Vitamin B12 level may be significantly lower im Megaloblastic anemia [21]

Only minority of patient with MCV level above 100fl are deficient with vitamin B12 or folate.[20] Patient with nonmegaloblastic macrocytic anemia have MCV less than 120cu microns. Alcohol ingestion is commonest cause of mild macrocytosis. Other causes of macrocytosis are liver diseases, severe hypothyroidism and chemotherapy induced [12]

In this study there are 9.18% cases with macrocytosis, in this case showed normal vit B12 and decreased or normal folic acid level

In treatment of IDA and vit B12 deficiency oral supplement or parenteral therapy can be given. [3,18]

**CONCLUSION:**

Result of this study demonstrates large number of iron deficiency anaemia cases seen in female with reproductive age group. In megaloblastic anaemia more number of cases are seen in age group between 50-60 years in both sex. For the diagnosis of microcytic hypochromic anemia haemogram by automated hematology analyzer, PBS examination serum iron profile is necessary for correct diagnosis and for macrocytic anemia haemogram, PBS examination, bone Marrow aspiration cytology and assay of two vitamins like B12 and folic acid and reticulocyte are needed for diagnosis. Serum iron profile, vit B12, folic acid and bone marrow aspiration should be done in addition of PBS examination for correct diagnosis and treatment of nutritional anemia.

**ACKNOWLEDGEMENTS:**

**FUNDING: No funding**

**COMPETING INTERESTS: No**

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**FIGURE WITH LEGENDS**

**TABLES:**

**Tables:**

**Table 1 : Age and sex distribution of anaemia in study;**

|  |  |  |  |
| --- | --- | --- | --- |
| Serial number | Age group in years | Male | Female |
| 1 | 20-29 | 22(8.42%) | 73(27.96%) |
| 2 | 30-39 | 18(6.89%) | 40(15.32%) |
| 3 | 40-49 | 24(9.19%) | 18(6.89%) |
| 4 | 50-60 | 32(12.26%) | 34(13.02%) |
| Total |  | 96(36.78%) | 165(63.21%) |

**Table 2 : Distribution of cases according to age groups in microcytic hypochromic anaemia:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Age group in years** | **Total number of cases with microcytic hypochromic anaemia** | **Iron deficiency anaemia** | **Anaemia of chronic disease** | **Haemoglobin deficiency** |
|  | Male | Female | Male | Female | Male | Female | Male | Female |
| 20-29 | 11 | 65 | 04 | 54 | 05 | 7 | 02 | 4 |
| 30-39 | 10 | 32 | 03 | 26 | 04 | 4 | 03 | 2 |
| 40-49 | 05 | 11 | 02 | 04 | 03 | 4 | 00 | 3 |
| 50-60 | 10 | 21 | 02 | 10 | 05 | 7 | 03 | 4 |
| Total | 36(13.79%) | 129(49.42%) | 11(4.21%) | 94(36.01%) | 17(6.51%) | 22(8.42%) | 08(3.06%) | 13(4.98%) |

**Table 3 : Distribution of cases according to age group in macrocytic anaemia :**

|  |  |  |  |
| --- | --- | --- | --- |
| **Age group in years** | **Total number macrocytic anaemia** | **Megaloblastic anaemia** | **Nonmegaloblastic macrocytic anaemia** |
|  | Male | Female | Male | Female | Male | Female |
| 20-29 | 11 | 08 | 07 | 06 | 04 | 02 |
| 30-39 | 08 | 08 | 04 | 07 | 04 | 01 |
| 40-49 | 19 | 07 | 14 | 07 | 05 | 00 |
| 50-60 | 22 | 13 | 17 | 10 | 05 | 03 |
|  | 60(23.98%) | 36(13.78%) | 42(16.09%) | 30(11.49%) | 18(6.89%) | 06(2.29%) |

**Table 4 : Clinical features of microcytic hypochromic anaemia**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr no** | **Symptoms** | **Number of cases** | **Signs** | **Number of cases** |
| 1 | Fatigue, palpitation, weakness | 68 | Pale conjunctiva, pale tongue and pallar  | 83 |
| 2 | Menstrual bleeding | 38 | Angular stomatitis | 12 |
| 3 | Urinary tract infection | 12 | Chronic renal disease | 18 |
| 4 | Gastrointestinal bleeding, fresh blood in stool | 15 | Lower respiratory tract infection  | 23 |
| 5 | Chronic respiratory infection | 11 | Haemorrhoids, GI malignancy, maleana | 10 |
| 6 | Post-menopausal bleeding | 14 | Growth endometrium/cervix | 19 |
| 7 | Joint pain | 12 | Rheumatoid arthritis | 08 |
| 8 | Dyspnea on exertion | 28 | DUB | 32 |

**Table 5 : Clinical History-Macrocytic anaemia**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr no** | **Symptoms** | **Number of cases** | **Signs** | **Number of cases** |
| 1 | Lethargy | 32 | Glossits  | 10 |
| 2 | Tingling and numbness | 18 | Pallar | 2 |
| 3 | Abdominal pain | 12 | Angularcheilosis | 12 |
| 4 | Fever | 08 | Sleenomegaly | 04 |
| 5 | Weightloss, loss of appetite | 19 | Hepatomegaly | 03 |
| 6 | Alcoholism | 14 | Neural manifestation-Myopathy | 05 |
| 7 | Pure vegetarian | 07 | Ecterus | 03 |
| 8 | History of haemolytic anaemia | 06 |  |  |