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Study of Iron Deficiency Anemia in Patients with Low Hemoglobin Level Attending Birat Medical College Teaching Hospital, Nepal

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ABSTRACT

Introduction: Despite nutritional advancements and iron fortification programs, anemia remains the top global health burden for decades. Serum ferritin level is one of the important adjunct diagnostic methods or the diagnosis of iron deficiency anemia.

Objectives: To study the prevalence of iron deficiency anemia in anemic patients, to categorize the different types of anemia based on peripheral blood smear findings and to observe serum ferritin level in all anemic patients.

Methodology: This was a hospital based prospective cross-sectional study carried out in Birat Medical College Teaching Hospital where 350 anemic patients were included. All anemic patients of either sex, aged 20 years or older, and had hemoglobin levels below normal for their respective sexes were included in the study. Similarly, children below twenty years who were anemic, patients with blood cancers (acute or chronic leukemia) and those with chronic inflammation and liver disease were excluded as these conditions will lead to elevation of serum ferritin level.

Results: Out of 350 anemic patients, the overall prevalence of iron deficiency anemia in anemic patient was found to be 37.1% based on serum ferritin level. According to the morphology of cells on peripheral blood smear, it was seen that 183 (52.2%) patients had microcytic hypochromic anemia, 33.4% had normocytic normochromic anemia and 14.3% patients had macrocytic anemia. Similarly, iron deficiency anemia was found to be more common in females (38.6%) than in male patients (34.4%) with 21-30 years age group most commonly affected.

Conclusion: Microcytic hypochromic anemia was the most common morphological type of anemia with the iron deficiency anemia being the commonest one based on serum ferritin level.

Introduction

Despite nutritional advancements and iron fortification programs, anemia remains the top global health burden for decades.[1] The prevalence of anemia is 48% in preschool-age children (less than 5 years of age), 25% in school-age children (5 to 14 years), 13% in males (15 to 59 years), 42% in pregnant females, 30% in women of reproductive age (15 to 49 years), and 24% in the elderly (>60 years)according to the statistics of the World Health Organization (WHO).[2]

Anemia is a major public health problem in the worldwide with prevalence of 43% in developing countries and 9% in developed nations.[3] Anemia is described as a clinical condition characterized by a decrease in total circulating red cell mass below normal limits or a decrease in blood hemoglobin concentration in comparison to normal values for that age and sex.[4] Individuals at any stage of life can be affected, although lactating and pregnant women, growing infants and

the elderly are most susceptible, which may increase the risk of impaired cognitive and physical development and increased mortality and morbidity rate.[5] In spite of its multifactorial etiology, anemia might be nutritional (iron, folic acid, and vitamin B12), inherited (thalassemia and sickle cell), environmental pollutants (lead), infectious (malaria), socioeconomic (low maternal level of education and low household income), demographic factors (age and gender), autoimmune (hemolytic anemia), malabsorption (achlorhydria), and chronic (chronic renal failure ,rheumatoid arthritis, tuberculosis, cancer).[6]

Iron deficiency anemia (IDA) has been reported to affect about 50-60% of young children and pregnant females as well as 20-30% of non- pregnant females in the developing countries.[7] This high rate might be due to increased iron demand/ loss or decreased iron intake, high nutritional needs during childhood and pregnancy, chronic intestinal blood loss due to parasitic and malarial infestations, iron malabsorption (celiac disease).[3] Serum ferritin level is one of the important adjunct diagnostic method for the diagnosis of IDA.[8]

Most of the previous studies on anemia are conducted on school children and pregnant women, yet there is little information about the prevalence of IDA in a generalized population.

Hence this study might be helpful to know the prevalence of IDA in a generalized population in our setting. This study aims to determine the prevalence of IDA among anemic patients, characterize the morphological types of anemia using peripheral blood smear examination and to correlate these findings with serum ferritin levels.

Methodology

This was a hospital based cross sectional study carried out in the department of Pathology, Birat Medical College Teaching Hospital. An ethical clearance for the study was obtained from the Institutional Review Committee (IRC) with IRC-PA-370/2024 of the institute. Patients who were referred to the Laboratory of Birat Medical College Teaching Hospital were anemic patients of either sex, aged 20 years or older, and had hemoglobin levels below normal for their respective sexes (for males, less than 13 gm/dl, and for females, less than 12 gm/dl).[8] During the study period, a total of 350 anemia patients were included in this study. Consecutive sampling technique was used to collect he sample. The sample size was calculated using the formula $n=z^2pq/d^2$,=1.962x0.35x0.65/0.0052=349.6=350

Where, z= standard normal variant for level of significance 0.05 (CI=95%), p= expected proportion in population based on previous study done in Bhairahawa is 35%7, q=1-p, d= absolute error or precision= 5%.

Children under twenty years old who had inherited blood disorders, chronic systemic illness and patients with blood cancers (acute or chronic leukemia) were excluded. Following aseptic precautions, preliminary blood tests including complete blood count and red cell indices were performed on 5ml venous blood. A completely automated analyzer (Yumizen H550: HORIBA) was used to measure full blood counts and red cell indices. Leishman stained blood film was made for every patient and examined. Further, serum ferritin estimation was performed using the competitive ELISA method. The peripheral blood film (PBF) was evaluated by a pathologist after reviewing the complete blood count (CBC) and serum ferritin level. A serum ferritin concentration of <15 ng/ml was considered diagnostic in both sexes.[8]

The data were entered into Microsoft Excel version 2007and then transferred to statistical package of social science (SPSS) version 16 for the analysis. The data were analyzed using frequencies and percentages and significant difference was tested using independent sample t-test.

Results

Three hundred and fifty anemic patients were included in this study. The age group most commonly affected in anemic patients was 21-30 years (n=76;21.7%) followed by 71-80 years age group (n=69;19.7%) with the mean±SD (52.63±20.47). (Table 1)

Table 1: Age distribution of anemic patients (n=350)

Age group (years)	Normocytic normochromic anemia (%)	Macrocytic normochromic (%)	Microcytic hypochromic anemia (%)		Total anemic patients (%)
			With normal ferritin	With decreased ferritin	
21-30	16	11	9	40	76 (21.7)
31-40	16	6	5	17	44 (12.6)
41-50	11	2	11	12	36 (10.3)
51-60	22	7	5	14	48 (13.7)
61-70	17	12	16	17	62 (17.7)
71-80	26	9	7	27	69 (19.7)
>80	9	3	0	3	15 (4.3)
Total	117 (33.4)	50 (14.3)	53 (15.1)	130 (37.1)	350

The overall prevalence of IDA was more common in females (n=228;65.1%) than in male patient (n=122;34.9%). Similarly, iron deficiency anemia was also more common in females (n=88;38.6%) than in male patients (n=42;34.4%).(Table 2)

Table 2: Sex wise distribution of IDA in anemic patients (n=350)

Sex	Normocytic normochromic anemia (%)	Macrocytic normochromic (%)	Microcytic hypochromic anemia (%)		Total anemic patients (%)
			With normal ferritin	With decreased ferritin	
Male	43(35.2)	18(15.6)	19(14.8)	42(34.4)	122 (34.9)
Female	74(32.5)	32(14.9)	34(14)	88(38.6)	228 (65.1)
Total	117	50	53	130	350

According to the morphology of cells on peripheral blood smear, it was seen that 183 (52.2%) patients had microcytic hypochromic anemia, 33.4% had normocytic normochromic anemia and 14.3% patients had macrocytic anemia. (Table 3)

Table 3: Red cell morphology of anemic patients based on peripheral blood smear (n=350)

Morphological classification of anemia	Number of patients	Percentage (%)
Normocytic normochromic	117	33.4
Microcytic hypochromic	183	52.2
Macrocytic normochromic	50	14.3
Total	350	100

Among 183 microcytic hypochromic anemic patients, 37.1% had decrease serum ferritin level while 15.1% had microcytic hypochromic anemia with normal serum ferritin level. Therefore, the overall prevalence of iron deficiency anemia in anemic patients was 37.1% cases. (Table:4)

Table 4: Distribution of Serum ferritin in anemic patients (n=350)

Ferritin (ng/ml)	Normocytic normochromic anemia (%)	Macrocytic normochromic (%)	Microcytic hypochromic anemia (%)		Total anemic patients (%)
			With normal ferritin	With decreased ferritin	
<15	0(0)	0(0)	0(0)	130(100)	130 (37.1)
15-300	117(53.2)	50(22.7)	53(24.1)	0(0)	220 (62.9)
Total	117 (33.4)	50 (14.3)	53 (15.1)	130 (37.1)	350

Significant difference was tested between iron deficiency anemia versus non-iron deficiency anemia (normocytic normochromic anemia, macrocytic normochromic anemia and microcytic hypochromic anemia). Among them there was no significant difference found between IDA and non-IDA with Hb (p=0.573) and MCV (p=0.766), however, serum ferritin was found highly significant (p<0.001). (Table 5)

Table 5: Independent sample t test of variables in study (n=350)

Variables	Anemia	Number	Mean±SD	p-value
Hb (gm/dl)	IDA Non-IDA	130 220	8.45±1.98 9.26±1.86	0.573
MCV (FL)	IDA Non-IDA	130 220	73.33±11.35 82.4±11.23	0.766
Ferritin (ng/ml)	IDA Non-IDA	130 220	7.19±3.63 111.1±94.53	<0.001

Discussion

Serum ferritin estimation has emerged as an effective noninvasive technique for the diagnosis of IDA as it assesses the body iron stores.

In this study of 350 anemic patients, we analyzed morphological classifications, and biochemical confirmation of IDA, comparing our findings with other published studies. Age distribution among anemic patients, showed that anemia was more prevalent in the age group 21-30 years (21.7%) followed by 71-80 years age group (19.7%). In a similar study conducted by Mishra et al, 42.5% cases were seen in 21-40 years age group.[7] However, in a study done by Kim et al, it was seen that the prevalence of anemia was more common (12.3%) in men of 70 years and older.[10] The younger age group affected in our study can be due to prevalence of hookworm infestation in our locality as well as lack of public awareness regarding healthy diet rich in iron content. Similarly, elderly male was more commonly affected due to increasing rate of inflammatory bowel disease in this age group.

In the present study, iron deficiency anemia was observed to be more common in females (38.6%) than in males (34.4%). Although no statistical test was performed to establish the significance of this difference, a similar trend has been consistently reported in previous studies. Mishra et al. reported a prevalence of 42.8% in females, while Al-Alimi et al. observed an even higher prevalence of 54%.[7,3] This female predominance may be explained by the increased physiological demands of iron during pregnancy and lactation, as well as blood loss associated with menstruation and

childbirth.[7,3] This can be due to increased demand of iron during pregnancy, lactation and during labor.

According to the morphology of cells on peripheral blood smear, it was seen that 183 (52.2%) patients had microcytic hypochromic anemia, 33.4% had normocytic normochromic anemia and 14.3% patient had macrocytic anemia. Similarly, in a study conducted by Chausaria et al and Mishra et al, microcytic hypochromic anemia was the most common anemia based on morphology of cells on peripheral blood smear with the percentage being 72% and 47%.[4,7] Microcytic hypochromic anemia is most common type of anemia globally primarily because it's leading cause, iron deficiency, is exceptionally widespread. Also, iron is essential for hemoglobin synthesis, its deficiency arises from a broad array of very common dietary, physiological and pathological causes.

In our study, out of 52.2% cases of microcytic hypochromic anemia, 37.1% cases were having IDA which was confirmed by measuring serum ferritin level of the patients and the remaining 15.1% cases were having microcytic hypochromic anemia with normal serum ferritin level. Normal serum ferritin in 15.1% cases with microcytic hypochromic anemia can be due to other causes of microcytic hypochromic anemia such as thalassemia, sideroblastic anemia and lead poisoning. Serum ferritin was also performed in macrocytic and normocytic normochromic anemia patients and results were found within normal range. Ozdemir et al have shown that iron level and total iron binding capacity fluctuate with age and dietary status of the patients.[11] Hence, peripheral blood smear and serum ferritin estimation in anemic patients were done in our studyand significant difference between the means of two variables were analyzed by independent sample t-test which was found to be statistically significant ($p<0.001$). There was no significant difference found between IDA and non-IDA with Hb ($p=0.573$) and MCV ($p=0.766$) which can be due to heterogeneity in non-IDA groups such as thalassemia, sideroblastic anemia, hemolytic anemia where hemoglobin reduction can be similar. Likewise, MCV values in IDA and non-IDA often fall in the same range reducing the statistical separation.

In this study, the overall prevalence of iron deficiency anemia in anemic patients was 37.1%.In a similar study conducted by Sinha et al, Patel et al, Kafle et al and Lamsal et al, the prevalence of IDA was found to be 25.57%, 40%, 49% and 41.35% respectively.[12,13,14,15] IDA is more common as it becomes symptomatic earlier as body recycles iron efficiently but if there is a sudden loss or increased demand, stores can deplete rapidly while other deficiencies like B12 takes longer to develop because the body stores several years' worth in the liver. It is necessary to diagnose IDA in early stage so that the underlying cause can be identified and treated on time, to support healthy pregnancy outcome and to prevent serious cardiovascular complications.

Conclusion

There is lack of recent and localized data on prevalence of IDA and it's differentiation from other anemia in our specific patient population. This study employs an integrated approach, utilizing PBS morphology as a primary classifier followed by serum ferritin confirmation, and to provide a precise and cost effective epidemiological and diagnostic analysis.

Recommendations

Future research should include a larger, multicenter sample and a complete iron profile, along with bone marrow iron studies when indicated, to better characterize anemia subtypes and guide appropriate interventions

Limitations of the Study

The study was single-centered with a modest sample size, and iron status was determined solely by ferritin measurement. Ferritin is an acute-phase reactant and may be elevated in inflammatory states, potentially masking iron deficiency. More comprehensive iron profiling (including serum iron, total iron-binding capacity, transferrin saturation, and soluble transferrin receptor levels) and bone marrow iron staining would allow more accurate classification.

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