THE PREVALENCE AND CLINICAL IMPACT OF LYMPHOCYTOPENIA IN PATIENTS WITH IRON DEFICIENCY ANEMIA AND THEIR RESPONSE TO IRON THERAPY IN CHIKKAMAGALURU DISTRICT, KARNATAKA

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ABSTRACT

The link between iron deficiency anemia (IDA) and lymphocytopenia is not well established in the literature. This study aims at assessing the prevalence and clinical impact of lymphocytopenia in IDA patients considering the impact of iron replacement on the total and differential WBCs count. ***Subjects and Methods:*** The records of all female patients with IDA who attended our Hematology Central Laboratory (April 2022 to April 2023) were retroprospectively reviewed. Age, CBC, and iron parameters were collected before and after treatment. (IV iron therapy and oral iron). ***Results:*** Out of 103 adult females with IDA, 48 patients had lymphocytopenia (46.6%). After iron therapy, their mean lymphocytes increased significantly towards normal range. However, no significant correlation was found between leukocytes, Neutrophil count with Serum ferritin.

Conclusions: Lymphocytopenia may occur in patients with IDA and are reversible with iron therapy. Iron therapy led to the correction of anemia in 100% and increased lymphocytes count in all 46.6% patients.Therefore, lymphopenic women with IDA should be treated, initially only with iron, and observed for their Hb and lymphocyte count responses before starting any other treatment.

ARTICLE

INTRODUCTION

Iron deficiency anemia (IDA) is one of the most common nutritional anemia, affecting globally, where adult females of childbearing age and children are among the most affected groups[1]. Most common etiology is found to be dietary deficiency followed by impaired absorption as in gastrectomy patients, growing infants, pregnancy, lactation and chronic blood loss from Gastrointestinal tract.[2] Source of blood loss could be from menorrhagia, uterine cancer, hemorrhoids, hookworm infestation or NSAIDS. Iron is necessary for cellular immune responses, oxidative metabolism within the mitochondria and production of hemoglobin and myoglobin. IDA causes an extensive spectrum of signs and symptoms as well as short and long-term complications[1]. Many studies have shown that iron deficiency is associated with a longer length of stay in hospital, cognitive dysfunction, increased risk of falls and reduced life expectancy. Paradoxically, iron is one of the most abundant minerals on earth and is plentiful in all but the most restricted diets.[1] Around 14% of all women could experience excessive or irregular menstrual bleeding, which can lead to varying degrees of IDA[3]. Furthermore, IDA might be associated with chronic conditions such as cancers, kidney, cardiovascular, and gastrointestinal diseases etc [4, 5].

The usual approach of assessing anemia by measuring only hemoglobin levels lacks both specificity and sensitivity. The World Health Organization (WHO) expert committee have come up with a more comprehensive definition which including several factors such as gender, age, ethnicity, altitude and based on statistical distribution considerations and cut-off points[4]. It is advised that "a Hb concentration below 13.7 g/dL in a white man, aged between 20 and 60 years, would have only an approximately 5% chance of being a normal value. For older men, this Hb value would be 13.2 g/dL. The corresponding value for women of all

ages would be 12.2 g/dL"[6]. Few studies have been investigating possible effects of IDA on white blood cells and the clinical impact of WBCs in response to iron therapy. Occurrence of IDA at high altitudes is still controversial. But additional problem when correcting hemoglobin at high altitudes is that the frequency of erythrocytes is decreased.

This study aims at evaluating the possible association between IDA and lymphocytopenia in adult non pregnant females and the clinical impact of iron therapy on lymphocyte counts. We also explored the association between deranged WBC and occurrence of infections in these patients, before and during iron replacement therapy. To the best of my knowledge, this is the first study to address this association among chikkamagaluru female population.

MATERIALS AND METHODS

A retroprospective observational study has been conducted at Aralaguppe Mallegowda District Hospital,Chikkamagaluru, Karnataka (including 8 Community Health Centres). All methods were performed in accordance with standard guidelines. The electronic health records of all female patients attending Hematology Central Laboratory (from April, 2022- April, 2023) with a diagnosis of IDA have been reviewed. The diagnosis was established based on the hemoglobin level along with iron parameters. Inclusion criteria included all females of child bearing age, aged 18-45 years, with confirmed IDA. Exclusion criteria consists of patients with other possible causes of anemia such as hemoglobinopathies, vitamin B12 or Folic acid deficiency, pregnancy, any chronic disorders and any concurrent drug use.

The following data were collected: age, complete and differential blood counts and iron parameters before and after intravenous iron therapy. Associated infections who had IDA and lymphocytopenia were noted, including the course of infections

and the response to treatment. Lymphocytopenia was diagnosed when lymphocytes count was less than 1.5 $x10^9$ /L. Parenteral iron therapy was given to all patients who had severe symptomatic anemia, bleeding and non-tolerance to oral iron therapy. Intravenous iron therapy of Iron sucrose given to required patients based on the formula: Total iron deficiency in mg= 2.3*Body weight*(Target Hb- Patient Hb)[7].

RESULTS

Out of 103 patients with IDA, 48 patients had lymphocytopenia (46.6%). Their mean lymphocyte count increased from 1.81 ± 0.15 x 10^9 /L before iron replacement therapy to 3.20 ± 0.75 x 10^9 /L after iron therapy (Table 1). 6 out of 48 patients presented with complaints of infections (3 with Upper respiratory tract infections, 2 with eczema and 1 with gastroenteritis).

Out of 103 females with IDA (Mean age: 36 ± 5 years), 60 patients had leukopenia (58.3%) and 5 patients had ANC less than 1.5 (4.8%). Their mean leukocyte count was 3.99 ± 0.64 x 10^9 before iron replacement therapy. After the correction of anemia, the leukocyte count increased significantly to 6.08 ±1.57 x 10^9 /L ($p<0.05$) (Table 1).

All 5 patients who had neutropenia contracted an infection at the time of presentation: 1 with upper respiratory tract infection, 2 with cervical lymphadenitis and 1 with viral fever. All of these patients received antibiotics without any reported complications. After iron therapy, 1 patient show increase in ANC but all 4 patients did not show any change.

Hb concentration did not show significant change between the two groups but ferritin levels show significant change between the two groups. Mean ferritin

levels before iron therapy was 13.22 ±0.5 ng/ml but it show significant increase to 67.5 ± 1.15 ng/ml after iron therapy ($p<0.05$) (Table 1).

DISCUSSION

IDA is a current issue all over the world. In the Foothill of Western Ghat region (Kadur, Birur, Sringeri, Koppa, NRPura, Panchanhalli, Tarikere, Mudigere), the prevalence of IDA in women appears to be considerably high, ranging between 14 to 67%.

In our study, IDA was reported in 31% of unmarried women and 69% of married (otherwise healthy) women. Another study was done by Dr. Puttaraju and Dr. H.R.Uma in Mysuru district of Karnataka which showed that out of 248 females, 35.4% were found to be suffering from anemia. This shows that Anemia is still a major health problem in Karnataka. According to Indian Health Report on Nutrition, 62.5% of women aged 15-49 years and 51.3% of adolescent girls aged 15-19 years are anemia, which is again higher than the all India level of 55.3% and 55.8% for the age group respectively.[8]

In this study, it is also noted that 5% of IDA women had neutropenia. In contrast to our study, Denic S et al, studied 1032 adult Arab participants in Saudi Arabia and reported that 10.7% had neutropenia $<1.5 \times 10^9$ /L.[9]

The impact of Iron deficiency and its relationship with the occurrence of neutropenia, lymphocytopenia and leukopenia has not been well established. In this study, Lymphocytopenia and Neutropenia was present in 46.6% and 4.8% respectively. It is also seen that 58.3% patients show decrease in their total count. The iron replacement was associated with significant increase in lymphocyte counts in all 100% of patients and increase in Hb in 50.5% of patients. Also there is an increase in total count after iron replacement therapy in 95% of patients.

Although maximum patients with IDA respond to Intravenous Iron Replacement Therapy, there are few patients especially childrens and teens, who suffer from Iron Refractory Iron Deficiency Anemia(IRIDA) who don't respond to the therapy. IRIDA.[10] is a rare recessive condition resulting from mutations of *TMPRSS6*, [11]leading to an inability to cleave the BMP coreceptor HJV and inhibit hepcidin.

Iron is an important factor in many pathways and the effects of iron deficiency on bone marrow functions other than erythropoiesis are not well defined. It was suggested that iron deficiency could impair enzymes that are involved in leukopoiesis[1]. Several reports have shown a correlation between immune cell function and iron concentration. Low iron levels may lead to macrophage and neutrophil dysfunction. "Iron levels have also been shown to alter
the proliferation of TH1 and TH2 subsets, likely related to the difference independence of cells on transferrin related iron uptake".[12,13]
Furthermore, iron is an essential nutrient for the growth of various pathogens.[14] During infections, the availability of iron affects both the pathogen proliferation and efficacy of antimicrobial immune pathways.
In this study, 6 out of 48 patients had IDA with lymphocytopenia(46.6%) and 5 patients out of 103 had IDA with decreased ANC(4.8%). Patients who were having IDA with lymphocytopenia presented with an infection like upper respiratory tract infection, eczema and gastroenteritis) at first examination.All were treated commonly without causing any severe complications.
Our study shows some limitations because it did not include any males as IDA is more commonly seen in females as compared to males considering the imbalaced analysis as the males sample size would be smaller.Additionally, this is a complete observational study. Therefore, further studies, usually the controlled studies are required to confirm the findings in this study.

CONCLUSION

In our study, lymphocytopenia occurred in 46.6% of patients with IDA. Iron replacement therapy led to the correction of anemia and increased lymphocyte count in 100% of patients. It is also noted the infection has been subsided after the replacement therapy. Therefore, lymphocytopenic women with iron deficiency anemia should be treated, initially only with iron, and observe for the infection, their Hb and ANC responses before starting any other treatment.

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Table 1: Blood parameters of patients with IDA before and after therapy

Before treatment	**WBC**	**Hb**	**MCV**	**ANC**	**LYMPHOCYTE**	**Serum iron**	**TS**	**TIBC**	**Serum Ferritin**
Mean	3.99	9.18	71.17	2.9	1.81	6.73	8.19	82.09	13.22
SD	1.62	2.51	9.18	1.36	0.93	6.54	3.01	8.5	7.74
AFTER TREATMENT									
Mean	6.08□	10.15□	82.58□	2.92	3.2□	20.73□	18.88□	50.19□	67.5□
SD	1.34	1.78	5.06	1.34	0.68	9.1	3.82	8.81	61.2

Legend: □P<0.05; White blood cell count(WBC): 4.5-11.0 x 10^9 /L; Hemoglobin(Hb): 12.5-16g/dL; MCV:80-100fl(90fl); ANC: >1.5 x 10^9 /L; Lymphocytes: 1.5-4.5 x 10^9 /L; Total serum iron: 4.6-30.4 µmol/L; Iron(transferrin) saturation: 20.8-41%, Total Iron Binding Capacity: (45-81) µmol/L; Serum Ferritin: 3.0-6.0ng/ml.[15]