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# The Role of Iron Profile in the Differential Diagnosis of Microcytic Hypochromic Anemia in King Abdulaziz Medical City

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## ABSTRACT

**Introduction:** Anemia is an abnormal decrease in red blood cells (RBCs) and is classified as; macrocytic, normocytic or microcytic. Iron profiling along with complete blood counting (CBC) is performed to diagnose microcytic hypochromic anemia (MCA).

**Aims:** To evaluate the role of iron profile in differentiating MHA.

**Methodology:** Retrospective chart-review study conducted in 2019 on anemic patients attending King Abdulaziz Medical City. Values from CBC and iron profiling tests were collected.

**Results:** 219 patients with MHA, 164 females and 55 males, were identified. Female patients were 74.9% of all cases, and adults were the most affected. Values of CBC parameters including hemoglobin, RBCs, packed cells volume (hematocrit), mean cell volume and mean cell hemoglobin were significantly lower in cases than controls ( $p < 0.001$ ). Iron profiling showed a significant association between all parameters and the diagnosis of iron deficiency anemia (IDA) and anemia of chronic disease, but not with TIBC. Iron profile values in thalassemia showed a significant association between diagnosis and serum iron and ferritin only. In sideroblastic anemia, iron profile values showed a significant association between all parameters except for transferrin.

**Conclusion:** IDA and anemia from chronic diseases are the highest among MHA. Iron profiling plays an important role in differentiating MHA.

**Key Words:** Anemia, Microcytic Hypochromic anemia, Thalassemia, Iron deficiency anemia, Anemia of chronic disease, Sideroblastic anemia, Complete blood count (CBC), Iron Profile test

## INTRODUCTION

Anemia is a very common condition and defined as an abnormal decrease in the total red blood cells (RBCs).<sup>1</sup> According to the world health organization, anemia affects 24.8% of the human population.<sup>2,3</sup> A recent study conducted on pregnant women indicated that 41.8% are affected.<sup>2</sup> According to a study conducted in Ethiopia, 11.5 percent of pregnant women were affected.<sup>4</sup> Another study of 1573 men, women, and children found that 13% of men and nearly half of women and children had iron deficiency.<sup>5</sup> Furthermore, a number of studies support the high prevalence of anemia.<sup>6,7,8,9</sup>

Anemia has several types and can be classified according to RBCs' morphology into three major classes.<sup>2,10,11,12</sup> Macrocytic anemia (large RBCs), normocytic anemia (normal RBCs size), and microcytic anemia (small RBCs).<sup>12</sup> Microcytic anemia is subdivided into hypochromic, normochromic, and hyperchromic in respect to the hemoglobin amount in the RBCs. Hemoglobin is a protein with four peptides responsible for RBCs redness and gas transportation, and it has iron as one of its important components.<sup>2,12</sup> Generally, anemia is a consequence of iron deficiency which leads to a decrease in hemoglobin production; however, pathogenesis may differ in some microcytic hypochromic anemia.<sup>1,12</sup> Thalassemia, for

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example, is a type of microcytic hypochromic anemia that is due to genetic mutation, while sideroblastic anemia is acquired.<sup>10,12</sup>

Iron profile test and complete blood count (CBC) are performed to diagnose anemia.<sup>11,12,13</sup> Iron profile test composed of serum iron test, total iron-binding capacity test (TIBC), serum ferritin test, and transferrin saturation test.<sup>14</sup> CBC contains several tests that are essential to determine cells morphology and pigmentation that are used to identify the anemia type, whether it is hypochromic or not, and whether it is microcytic or not.<sup>3,11,12,14</sup> Bone marrow examination and molecular tests are able differentiate microcytic hypochromic anemias.<sup>6,11,12</sup> In addition, a study used soluble transferrin receptor (sol TFR) test to diagnose anemia in patients with systemic lupus erythematosus, which showed better detection of iron deficiency anemia compared to iron profile test, and different studies have shown that CBC is essential in diagnosis and differentiation of anemias.<sup>6,7,13,15, 16</sup>

This study aims to evaluate the role of iron profile in the differential diagnosis of microcytic hypochromic anemias.

## MATERIALS AND METHODS

This is a retrospective chart review study conducted in anemic patients seeking consultation in King Abdulaziz Medical City (KAMC), Jeddah, Saudi Arabia. The study included 219 patients diagnosed with microcytic hypochromic anemia, (55 males and 164 females) in different age groups: 9 infants; 10 children; 9 teens; 148 adults; and 46 elderly. Results of CBC, red blood cell morphology, iron profile tests, demographic and clinical data were collected from the KAMC medical records after approval by the Institutional Review Board (IRB# SP19/327/J) at the King Abdullah International Medical Research Centre following the declaration of Helsinki.

### Data Management and Analysis Plan:

The data was collected in an excel sheet and entered into SPSS software (version 20). A backup soft copy version, as well as hard copy print, were dated, saved, and secured after each data entry. Categorical variables were presented in frequency and percentage distribution, while continuous variables were described by the mean and standard deviation (SD). Independent samples t-test and analysis of variance were conducted to examine significant differences between study variables; results with p-value less than or equal to 0.05 were set to be significant.

## RESULTS

Among the cases, 74.9% out of 219 were females, compared to 76% females out of 50 control individuals. Infants, children,

teens, adults and elderly accounted for 2.8%, 4.7%, 4.1%, 67.6%, 21% of the cases, respectively, with the highest percentage in adults. The Hb, RBC, PCV, MCV and MCH values were significantly lower in cases compared to controls ( $p < 0.001$ ). IDA was 40.2% in adults compared to 5.5% in the elderly and 1% in both infants and children (Table 1). ACD was 3.6% in both teens and infants, and 3.2% were children and 13.2% were elderly and 20.6% were Adults (Table 1). While thalassemia cases in adults were 5% and in children, it was 1%, and infant cases were only 0.5%. In contrast, sideroblastic anemia was present only in 2.3% of the elderly and 1.8% of adults (Table 1). IDA was the most common (49%) and 83.2% of them were females. ACD was identified in 40.6% (70.8% were females). In comparison, thalassemia cases were 6.5% and equally distributed between males and females. Sideroblastic anemia was the least common with 4.1% of all cases and 55.6% of them were females. Analysis of the iron profile outcome in IDA shows a significant association between all parameters and diagnosis (Table 2; Figure 1). While analyzing iron profile outcome in ACD shows a significant association between all parameters and diagnosis except for TIBC (Table 3; Figure 1). On the other hand, the analysis of the iron profile outcome in thalassemia shows a significant association between diagnosis and the serum iron and ferritin only (Table 4; Figure 1). In addition, the analysis of iron profile outcome in sideroblastic anemia shows a significant association between all parameters and diagnosis except for transferrin (Table 5; Figure 1).

## DISCUSSION

This study was conducted at KAMC-Jeddah with a sample size of 219 microcytic hypochromic anemic patients. Anemia is divided into different categories.<sup>10,11,12,13</sup> The microcytic hypochromic anemia is a condition with small, light-colored RBCs. The hypochromia and decrease in the RBCs size are due to defect in the hemoglobin synthesis.<sup>10,13</sup> The defect occurs as a result of the lack of globin product, abnormal iron metabolism, and abnormal synthesis of the heme group.<sup>10,13,17</sup> Generally, there are four types of microcytic hypochromic anemias; IDA, ACD, thalassemia, and sideroblastic anemia.<sup>10,11,12,13,17</sup>

The iron profile tests were performed in IDA patients, and the results showed a significant decrease in serum ferritin and transferrin saturation but a significant increase in TIBC compared to normal participants ( $p < 0.000$ ). IDA is a disease caused by the decrease of the iron level in the body that results in alteration of the production of hemoglobin amount since it is highly dependent on iron.<sup>17,18</sup> As a result, the RBCs produced are smaller and in lower numbers.<sup>17</sup> This explains the findings of the iron profile tests, in which all parameters were reduced except TIBC, which measures the free sites on transferrin where iron is bounded.<sup>18,19</sup>

ACD patients showed a significant decrease in serum iron and transferrin saturation ( $p$  0.000), but no significant change in TIBC. ACD is generally associated with inflammation and chronic infection, where the production of pro-inflammatory cytokines and the hepatic peptide hepcidin interfere with iron metabolism and absorption, affecting iron profile tests.<sup>20,21</sup>

In thalassemia patients, the serum ferritin and iron were significantly increased ( $p$  0.000) and ( $p$  0.025), respectively. Thalassemia is a genetic mutation that results in abnormal hemoglobin chains.<sup>22</sup> However, a study conducted on thalassemia trait patients showed that there was no significant difference in the serum iron.<sup>23</sup> The high ferritin level in thalassemia, which indicates iron overload, is believed to be the result of repeated blood transfusions.<sup>22</sup> In contrast, another study conducted on non-transfusion-dependent patients revealed that iron overload is a consequence of the increased absorption as a response to the low RBCs production.<sup>24</sup>

In sideroblastic patients, the iron profile showed a significant increase in serum ferritin ( $p$  0.000) and a significant decrease in serum iron and TIBC. Sideroblastic anemia is characterized by the accumulation of iron in the tissues as a result of a mutation in the gene that uses stored iron in the production of RBCs.<sup>25,26,27</sup>

Finally, female microcytic hypochromic anemia patients were 74.9% of all cases. The prevalence of IDA and ACD was significantly higher in female patients. A study suggested that the reason was the low educational level or pregnancy, while another study suggested menorrhagia.<sup>28,29</sup> Furthermore, in this study, microcytic hypochromic anemia was found in a wide range of adult age groups, whereas another study found the highest prevalence in elderly patients.<sup>30</sup>

### Limitations

One of the study's drawbacks is that it was based on a single-center; therefore, the study area and sample size need to be expanded to better understand the implication of iron profile in microcytic hypochromic patients. However, the study addressed some of the key approaches for assessing the status of the iron profile in patients with microcytic hypochromic anemia, which guide further work on the use of various diagnostic methods, including bone marrow, soluble transferrin receptors, and hemoglobin electrophoresis.

### CONCLUSION

Serum iron levels decreased significantly in all forms of microcytic hypochromic anemia, except in cases of thalassemia. TIBC was normal in all cases except IDA, where it was increased. Ferritin levels were significantly higher in thalassemia and sideroblastic anemia, whereas they were lower in IDA and normal in ACD. While transferrin saturation was only low in IDA. These findings highlight the importance

of iron profile parameters in differentiating and diagnosing microcytic hypochromic anemias. According to the findings of this study, iron deficiency anemia and anemia of chronic diseases have the highest prevalence among cases of microcytic hypochromic anemia.

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**Conflict of Interest:** None.

**Authors' Contribution:** MS and NSS designed and directed the project; RMA, AMA and GNM collected the data; MEA analyzed the data; NMA, ARS and AA interpreted the results. All authors contributed to manuscript writing and reviewing.

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**Table 1: The distribution of microcytic hypochromic anaemia types with respect to age groups.**

| Diagnosis | Infant% | Child% | Teens% | Adult% | Elderly% | p-value |
|-----------|---------|--------|--------|--------|----------|---------|
| ACD       | 1.8     | 3.2    | 1.8    | 20.6   | 13.2     | 0.001** |
| IDA       | 0.5     | 0.5    | 2.3    | 40.2   | 5.5      |         |
| Sidero    | 0       | 0      | 0      | 1.8    | 2.3      |         |
| Thala     | 0.5     | 1      | 0      | 5      | 0        |         |

\*significant at 5% \*\*significant at 1%

**Table 2: Comparison of iron profile parameters between patients with iron deficiency anemia and controls.**

| Parameter      | Mean cases | Mean control | p-value |
|----------------|------------|--------------|---------|
| Serum iron     | 4.5        | 16.4         | 0.000** |
| Serum ferritin | 3.4        | 54.2         | 0.000** |
| TIBC           | 89.6       | 56.6         | 0.000** |
| Transferrin    | 6.5        | 21.6         | 0.000** |

(Measuring units: µmol/l serum iron; µg/l serum ferritin; µmol/l TIBC; percentage Transferrin). \*significant at 5%, \*\*significant at 1%

**Table 3: Comparison of iron profile parameters between patients with anemia of chronic disease and controls.**

| Parameter      | Mean cases | Mean control | p-value |
|----------------|------------|--------------|---------|
| Serum iron     | 7.0        | 16.4         | 0.000** |
| Serum ferritin | 98.4       | 54.2         | 0.002** |
| TIBC           | 51.4       | 56.6         | 0.197   |
| Transferrin    | 17.1       | 21.6         | 0.028*  |

(Measuring units: µmol/l serum iron; µg/l serum ferritin; µmol/l TIBC; percentage Transferrin). \*significant at 5%, \*\*significant at 1%

**Table 4: Comparison of iron profile parameters between patients with thalassemia and controls.**

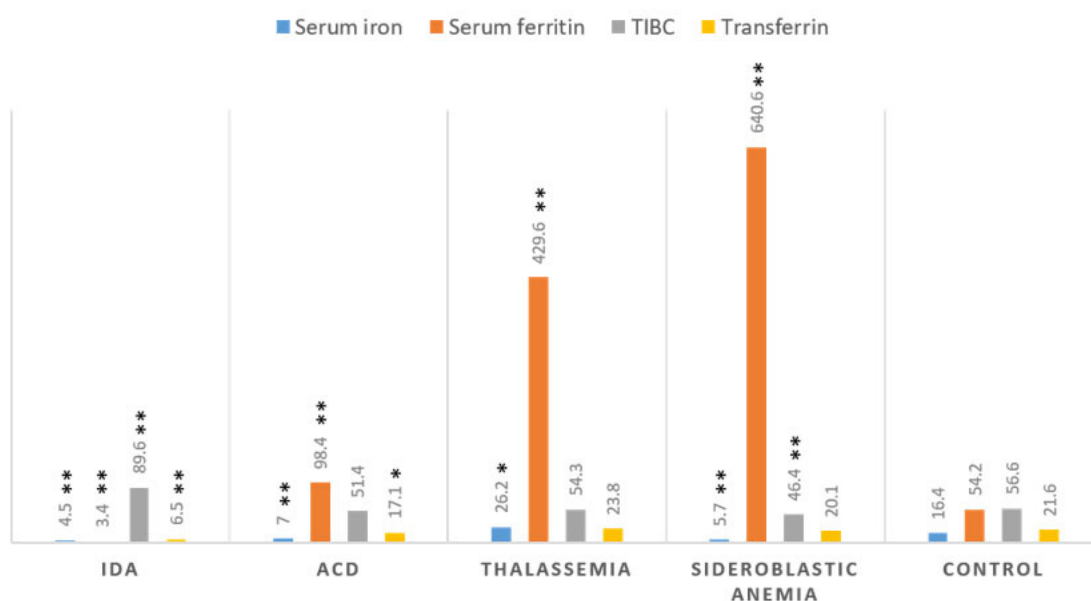
| Parameter      | Mean cases | Mean control | p-value |
|----------------|------------|--------------|---------|
| Serum iron     | 26.2       | 16.4         | 0.025*  |
| Serum ferritin | 429.6      | 54.2         | 0.000** |
| TIBC           | 54.3       | 56.6         | 0.473   |
| Transferrin    | 23.8       | 21.6         | 0.367   |

(Measuring units:  $\mu\text{mol/l}$  serum iron;  $\mu\text{g/l}$  serum ferritin;  $\mu\text{mol/l}$  TIBC; percentage Transferrin). \*significant at 5%, \*\*significant at 1%

**Table 5: Comparison of iron profile parameters between patients with sideroblastic anemia and controls.**

| Parameter      | Mean cases | Mean control | p-value |
|----------------|------------|--------------|---------|
| Serum iron     | 5.7        | 16.4         | 0.000** |
| Serum ferritin | 640.6      | 54.2         | 0.000** |
| TIBC           | 46.4       | 56.6         | 0.010** |
| Transferrin    | 20.1       | 21.6         | 0.605   |

(Measuring units:  $\mu\text{mol/l}$  serum iron;  $\mu\text{g/l}$  serum ferritin;  $\mu\text{mol/l}$  TIBC; percentage Transferrin). \*significant at 5%, \*\*significant at 1%



**Figure 1:** The mean of iron profile parameters with respect to the different types of microcytic hypochromic anemias. (Measuring units:  $\mu\text{mol/l}$  serum iron;  $\mu\text{g/l}$  serum ferritin;  $\mu\text{mol/l}$  TIBC; percentage Transferrin). \*significant at 5%, \*\*significant at 1%.