Research Article

Role of Hypochromia and Microcytosis in the prediction of iron deficiency anemia

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Abstract
Objective: The present study aimed at assessing the role of hypochromia and microcytosis in the determination of iron storage state and early detection of iron deficiency anemia (IDA). **Background:** Anemia is defined as hemoglobin (Hb) concentration below the reference range (Hb level below 13 g/dl in men, 12 g/dl in women, and 11g/dl in pregnant women and preschool children). The assessment of hypochromia and microcytosis can help more prediction of (IDA). **Patients and methods:** This study included 60 patients with normal Hb levels with decreased Mean Corpuscular Volume (MCV) & Mean Corpuscular Hemoglobin (MCH) and 30 healthy subjects as a control group. All patients were subjected to history taking, clinical examinations, laboratory investigations such as Complete blood count (CBC), serum iron, serum ferritin (SF), total iron-binding capacity (TIBC), C - reactive protein (CRP) and Hb. Electrophoresis. **Results:** Mean MCV, MCH, Serum iron, SF, TIBC, CRP, and Hb. Electrophoresis had statistically significant difference between the two groups (p-value <0.0001). **Conclusion:** It indicates an early sign of (IDA).
Abbreviations: IDA; iron deficiency anemia, Hb; hemoglobin, MCV; Mean Corpuscular Volume, MCH; Mean Corpuscular Hemoglobin, CBC; Complete blood count, SF; serum ferritin, TIBC; total iron-binding capacity and CRP; C - reactive protein.
Keywords: Anemia, hypochromia, and microcytosis.

Introduction
Anemia is defined as Hb concentration below the reference range[1]. Microcytosis means low mean corpuscular volume (MCV <80 fl) and hypochromia means low mean corpuscular hemoglobin (MCH <26 pg)[2]. There are four significant specific causes of microcytosis: IDA, anemia of chronic disorder, thalassemia, and sideroblastic anemia[3].

Iron deficiency anemia is chronic and frequently asymptomatic. Weaknesses, fatigue, difficulty in concentrating, and poor work productivity are non-specific symptoms[4]. The diagnosis of IDA is derived from a patient's clinical history and hematologic manifestations as reduced Hb, low MCV, and MCH[5]. Serum iron concentration is very low in IDA. Transferrin saturation (Tsat) level of less than 16% indicates an iron supply that is insufficient to support normal erythropoiesis[6,4]. Serum ferritin (< 30 ng/L) unequivocally means Iron deficiency. However, in the presence of an inflammatory process, iron deficiency could exist even with levels of ferritin up to 100 ng/mL[7]. Anemia of chronic disease (ACD) is a complication of chronic inflammatory diseases as cancer, rheumatoid arthritis, inflammatory bowel diseases, and congestive heart failure, sepsis, and chronic renal failure. Patients suffer from ACD when they have high CRP level, Hb concentration < 13 g/dL for male and < 12 g/dL for female and low TSat < 20%, but normal or increased SF concentration (> 100 ng/mL) or low SF concentration (30-100 ng/mL)[8].

Thalassemia is a genetic decrease in globin chain synthesis. There are many types of thalassemia and the most clinically relevant are the (alpha (α) and beta (β) thalassemia)[9]. In alpha thalassemia, the primary defect is the reduced or absent production of α globin chains, which constitute several Hb types, including HbA, HbF, and HbA2[10]. Beta thalassemia is hereditary blood disorders characterized by reduced or absent β globin chain synthesis, resulting in reduced Hb in red blood cells (RBCs), decreased RBCs production, and anemia[11]. The sideroblastic anemias are a heterogeneous group of inherited and acquired disorders characterized by anemia of varying severity and the presence of the ringed

sideroblasts in bone marrow[12]. Acquired sideroblastic anemias are either primary (myelodysplastic syndromes, refractory anemia with ring sideroblasts) or secondary (drugs, toxins, copper deficiency, or chronic neoplastic disease). Hereditary sideroblastic anemias are caused by defects in genes present on the x chromosome, autosomal chromosomes, or by defects in mitochondrial genes[13]. Sideroblastic anemia tends to be moderate to severe conditions with Hb (4 to10 g / dl). Red blood cells are usually microcytic, with low MCV and MCH and increased red cell distribution width (RDW). The diagnosis of sideroblastic anemia requires the presence of ringed sideroblasts in the bone marrow[14, 15].

Patients and methods

This study included 90 subjects; 60 patients with normal Hb level with decreased MCV & MCH selected from the outpatient clinics and inpatients in Al-Azhar University Hospitals - Assiut in the period from February to June 2016. In addition to 30, apparently healthy subjects of matched age and sex were chosen as a control group.

Exclusion criteria are anemic patients and patients decreased MCV & MCH with abnormal Hb electrophoresis. All patients were submitted to a full history taking, clinical examination and laboratory Investigations including CBC (using cell counter Micros 60-18P), serum iron (ferrozine colorimetric method endpoint), SF(enzyme immunoassay for the quantitative determination), TIBC (in-vitro quantitative diagnostic determination), CRP (by latex agglutination test), and Hb. Electrophoresis (agarose gel electrophoresis).

Statistical analysis

Statistical package for social sciences, version 17 Chicago, USA was used for data analysis. Data expressed as mean, standard deviation, number, and percentage. Mean and the standard deviation was used as a descriptive value for quantitative data. Using the t-test to determine the significance of numeric variables when comparing two groups. Using Chi-square, ANOVA, and post hoc to determine significance for non –parametric variables when comparing more than two groups. Using Pearson's correlation for the numeric variable in the same group. ($P > 0.05$ no significance) ($P < 0.05$ significant).

Results

Our results showed that there was a significant difference between the two studied groups as regards MCV, MCH, Serum iron, SF, TIBC. The comparison between the studied groups is given in detail in Table (1).

Table (1): Comparison between the studied groups regarding MCV, MCH, Serum iron, SF, TIBC

	Cases group	Controls group	P value
Hemoglobin **Range** **Mean ± SD**	 12-16 g/dl 13.5 ± 0.9 g/dl	 12.2-16 g/dl 13.8 ± 1 g/dl	0.195
MCV **Range** **Mean ± SD**	 70-83 fl 77.2 ± 2.99 fl	 81.9-96.3 fl 87.9 ± 4.4 fl	0.0001
MCH **Range** **Mean ± SD**	 22-28 pg 25.06 ± 1.22 pg	 27.5-32 pg 29.4 ± 1.2 pg	0.0001
Iron **Range** **Mean ± SD**	 3-165 µg/dl 34.6 ± 36.2 µg/dl	 76-165 µg/dl 112.4 ± 28.6 µg/dl	0.0001
TIBC **Range** **Mean ± SD**	 170-945 µg/dl 666.1 ±186.1 µg/dl	 298-418 µg/dl 369.3 ± 35.1 µg/dl	0.0001
Ferritin **Range** **Mean ± SD**	 1.1-452 µg/L IQR: 16.12-74.34 63.5 ± 82.7 µg/L Median: 38.4	 53-232 µg/L 129.3 ± 56.5 µg/L	0.0001

There was significant strong positive correlation in MCH with iron level in all patient groups (p=0.0001), significant moderate negative correlation in MCH with TIBC level (p=0.0001) and significant fair positive correlation in MCH with ferritin level (p=0.0001). Correlations of MCH with iron, TIBC and ferritin in all patients are given in details in Table (2).

Table (2): Correlations of MCH with iron, TIBC and ferritin in all patients.

	MCH	
	R	**P value**
Iron	0.784	0.0001
TIBC	-0.654	0.0001
Ferritin	0.463	0.0001

N.B.
r= 0- 0.24 (weak correlation)
r= 0.25- 0.49 (fair correlation)
r= 0.5- 0.74 (moderate correlation)
r= 0.75- 1 (strong correlation).

There was significant moderate positive correlation in MCV with iron level in all patient groups (p=0.0001), significant moderate negative correlation in MCV with TIBC level (p=0.0001) and significant fair positive correlation in MCV with ferritin level (p=0.0001). Correlations of MCV with iron, TIBC and ferritin in all patients are given in details in Table (3).

Table (3): Correlations of MCV with iron, TIBC and ferritin in all patients.

	MCV	
	R	**P value**
Iron	0.722	0.0001
TIBC	-0.598	0.0001
Ferritin	0.463	0.0001

Relative operating characteristics (Roc curve) was performed to assess the accuracy and specificity of ferritin with MCV and MCH in the diagnosis. Results of ROC curve of ferritin for prediction of low MCV and MCH in male and female are showed in Table (4).

Table (4): Results of ROC curve analysis of ferritin for prediction of low MCV and MCH

Ferritin	***MCV***							
	Sensitivity	**Specificity**	**AUC**	**St. error**	**Cut off value**	**P value**	**PPV**	**NPV**
(males)	100%	59.1%	0.833	0.0661	≤ 82	0.0001	60.9%	100%
(females)	100%	61.5%	0.816	0.0636	≤ 80	0.0001	66.7%	100%
Ferritin	***MCH***							
	Sensitivity	**Specificity**	**AUC**	**St. error**	**Cut off value**	**P value**	**PPV**	**NPV**
(males)	100%	59.1%	0.778	0.0785	≤ 27	0.0004	60.9%	100%
(females)	100%	65.4%	0.887	0.0456	≤ 26	0.0001	69%	100%

AUC (area under the curve)
PPV (Positive predictive value)
NPV (Negative predictive value)
ROC curve analysis of ferritin for prediction of low MCH and MCH in male and female are showed in Figure (1),(2),(3)and (4).

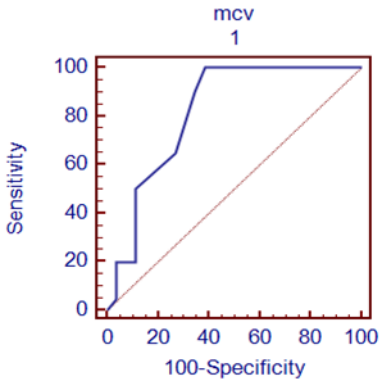

Fig. (1): ROC curve analysis of ferritin for prediction of low MCV in male.

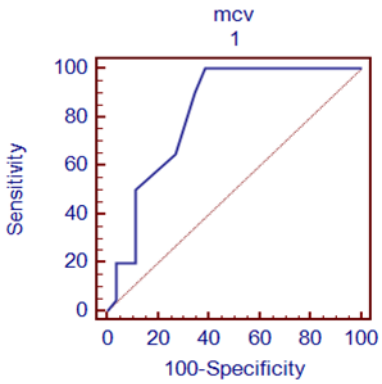

Fig. (2): ROC curve analysis of ferritin for prediction of low MCV in female.

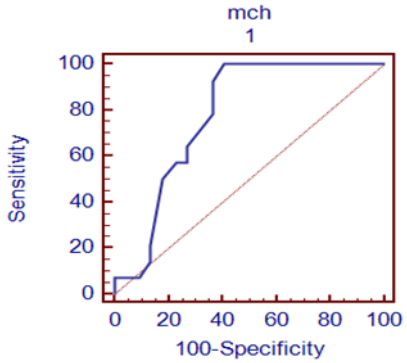

Fig. (3): ROC curve analysis of ferritin for prediction of low MCH in male

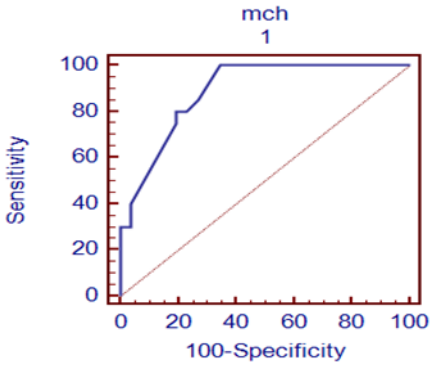

Fig. (4): ROC curve analysis of ferritin for prediction of low MCH in female.

Discussion

In this study, we use serum iron, TIBC, ferritin, CRP, and Hb electrophoresis for 60 patients who have normal Hb percent with decreased MCV & MCH. In addition 30 apparently healthy subjects of matched age and sex are chosen as the control group.

Hemoglobin in the control and case groups was quite close to each other. There was no significant value in Hb levels in these groups. It occurs due to the normal value of Hb in cases and controls. Our results are consistent with the results of Sindu and Ramakrishnan, (2016)[16] who make study in the diabetic group. Similar results found in the study of Ozluk et al., (2015)[17] who make study in patients diagnosed with angiographically documented peripheral artery disease.

In accordance with our selection criteria, MCH and MCV in our study were low in cases as compared to control. Similar results found in the study of Doganer et al., (2015)[18] who make a study about (Hb) levels correlates with the presence of coronary artery disease. Similar results found in the study of Manandhar and Bhattarai (2018)[19] who make a study to determine the prevalence of anemia in hospitalized patients. Similar results found in the study of Latif et al., (2017)[20] who make study in anemic patients with chronic kidney disease. However, this is in disagreement with the findings of Chen et al., (2017)[21] who make study in Alzheimer's group.

In the current study, we found that there was a highly statistically significant difference between the studied groups as regards serum iron, as it was lower in the case group than in the control group. This is in agreement with the findings of El Lehleha et al., (2017)[22] who found that anemia state was found in chronic hepatitis C patients. Similar results found in the study of Rubab et al., (2015)[23] who make a study in end-stage renal disease on hemodialysis. Similar results found in the study of Kunireddy et al., (2018)[24] who make a study in systemic lupus erythematosus patients. However, this is in disagreement with the findings of Muhsin et al., (2016)[25] who make study in the pre-eclamptic patients.

In this study, we found that there was a highly statistically significant difference between the studied groups as regards TIBC, as it was higher in the case group than in the control group. This is in agreement with the findings of Thakur and Guttikonda, (2017)[26] who make a study in oral submucous fibrosis patients. However, this is in disagreement with the findings of Nayak et al., (2017)[27] who make study in iron foundry workers.

In this study, we found that there was a highly statistically significant difference between the studied groups as regards (SF), as it was lower in the case group than in the control group. This is in agreement with the findings of Saha et al., (2016)[28] who make study in cases of febrile convulsion. Similar results found in the study of Sit et al., (2016),[29] who make study in cases of the first episode of simple febrile seizure. However, this is in disagreement with the findings of Sindu and Ramakrishnan, (2016)[16] who make study in diabetic cases.

Conclusion

The serum iron and serum ferritin levels in cases with hypochromia and microcytosis with normal hemoglobin were reduced and total iron-binding capacity increased as compared with controls. Results of C - reactive protein are negative and hemoglobin electrophoresis is normal. It indicates an early sign of iron deficiency anemia.

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