Utilization of the hematology autoanalyser as a screening test for iron deficiency anemia and β–thalassemia trait

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Abstract

Background: Hypochromic microcytic anemia is the most common encountered type of anemia and it includes several types, iron deficiency and β-thalassemia trait are the commonest examples, and since the definite diagnostic tests are not always available, therefore, the maximum utilization of the cell count autoanalysers for screening testing and reducing the number of patients who needs the use of more specialized techniques can be useful.

Objectives: To evaluate the results of hematology autoanalyser in iron deficiency anemia and β–thalassemia trait.

Patients & Methods: This is a cohort prospective study in which two hundred newly diagnosed patients with hypochromic anemia, and proved later to be either iron deficiency anemia alone or β–thalassemia trait alone are included. These patients were attending the National Center for Hematology from January 2009 to February 2010.

Results: There is a highly significant difference in red cell count, hemoglobin concentration and hematocrit percent (p-value < 0.001, 0.003 and < 0.001 respectively) between iron deficiency anemia and β–thalassemia trait. A highly significant relation is found between hemoglobin concentration and other red cell parameters and white blood cells and platelets count (p-value < 0.001 for all) in both types of anemia. Also a significant difference is noted between hemoglobin concentration and hematocrit percent to identify anemia in β–thalassemia trait (p-value 0.0236).

Conclusions: Red blood cells count is mostly in the lower normal threshold or below in iron deficiency anemia, and it is in the upper normal threshold or above in β–thalassemia trait. B–thalassemia trait usually presents with milder anemia or even with lower normal Hemoglobin concentration and hematochirt percent, while in iron deficiency the anemia is always clearly present. Relying on Hb concentration rather than on Hct percent to evaluate the presence of anemia makes its diagnosis more obvious. RDW higher than 24.3 % is only associated with iron deficiency anemia.

Keywords: Hematology auto analyzer, iron deficiency, thalassemia minor.

Introduction:

Anemia is a reduction of hemoglobin (Hb) concentration in peripheral blood below that expected for a healthy person of the same age, sex and physiological status [1].

The packed cell volume (PCV) or hematocrit (Hct) is an alternative way for detecting anemia. PCV refers to a measurement made after centrifugation and Hct for an estimate made by an automated instrument [2].

There is more than one system for classification of anemia, the one most commonly applied in practice is the morphological classification in which anemia is classified into three major categories, these are hypochromic microcytic, normochromic normocytic and normochromic macrocytic according to the mean cell hemoglobin (MCH) and mean cell volume (MCV) parameters [3].

Hypo chromic microcytic anemia is the most common encountered type of anemia and it includes several types of anemia, however, practically iron deficiency anemia and β-thalassemia trait are the commonest examples of hypochromic anemias [4].

The main red cell indices are MCV, MCH, mean cell hemoglobin concentration (MCHC) and red cell distribution width (RDW). These indices are the basis for classifying anemias, and in various combinations they have been used to aid in the distinction between different types of anemias including iron deficiency and β-thalassemia trait. Their use provides a guide to the most likely diagnosis, but they are not definite diagnostic tests [5].

In Iraq the definite diagnostic tests for iron deficiency anemia and β-thalassemia trait including serum iron, transferrin level and saturation and/or serum ferritin, and Hb electrophoresis or High performance liquid chromatography (HPLC) are not always available and even when some of them are available there is always the problem of inadequate supply of operating kits. (Therefore, the maximum utilization of the cell count autoanalysers for screening testing can be very useful and reduces the number of patients who needs the employment of more specialized techniques can be very useful .

Objectives:

To evaluate the results of hematology autoanalyser in iron deficiency anemia and β–thalassemia trait.

Patients, Materials & Methods:

This is a cohort prospective study in which two hundred newly diagnosed patients with hypochromic anemia, and proved later to be either iron deficiency anemia or β–thalassemia trait are included. These patients were attending the National Center for Hematology from January 2009 to February 2010.

Each patient was interviewed for personal, family history and physical examination according to our questionnaire.

For each patient the following tests were requested: Full blood count using the Abbott hematology autoanalyser CELL-DYN 1700 and peripheral blood smear (leishman stain). If the primary results revealed the presence of hypochromic anemia then the patient is asked to perform a serum iron, total iron binding capacity(randox) and transferrin saturation and hemoglobin electrophoresis by HPLC(biorad company) for confirmation of the initial diagnosis. Any patient proved to have either iron deficiency anemia alone or β-thalassemia trait alone is included in this study.

Patients who refused to cooperate with performing the confirmatory tests were excluded from the study. Patients with combined iron deficiency anemia and β-thalassemia trait or having other causes of hypochromic anemia were also excluded from the study.

Statistical analyses were performed by using Statistical Package for Social Sciences (SPSS; version 17.0) software. The results were expressed as mean ± standard error (SE), with p-value of less than 0.05 considered significant.

Results:

The sex and age distribution according to the type of anemia of the patients involved in this study are illustrated in **table 1.**

Male: female ratio is 1:1.5 in iron deficiency anemia, 1:1.9 in β–thalassemia trait and 1:1.6 as an overall sex ratio for both types of anemia.

The comparisons of the results of the hematology autoanalyser in iron deficiency anemia and β–thalassemia trait are presented in **table 2.**

The relations between Hb concentration and other red cell parameters in iron deficiency anemia and β–thalassemia trait are presented in **tables 3 and 4** respectively.

The relations between Hb concentration multiplied by 3 and Hct in iron deficiency anemia and β–thalassemia trait are presented in **tables 5.**

Table 1: Sex and age distribution of patients according to type of hypochromic anemia:

Parameter		Hypochromic anemia				Total	
		Iron deficiency		β -thalassemia trait			
		No.	%	No.	%		
Sex	Males	52	40	24	35	76	200
	Females	79	60	45	65	124	
Age	1-10	37	28	21	30	58	200
	11-20	30	23	13	19	43	
	21-30	24	18.5	9	13	33	
	31-40	12	9	8	12	20	
	41-50	17	13	8	12	25	
	51-60	11	8.5	10	14	21	
Total		131		69			
		200					

Table 2: Comparison of the results of the hematology autoanalyser in iron deficiency anemia and β–thalassemia trait:

Parameter	Iron deficiency anemia		β–thalassemia trait		P-value
	Range	Mean ± SE	Range	Mean ± SE	
RBC count (*1012/l) i	2.51-4.96	4.08 ± 0.13	4.64-6.21	5.47 ± 0.07	< 0.001
Hb concentration (g/dl) ii	5-12	8.29 ± 0.43	7.5-12.7	10.31 ± 0.3	0.003
Hematocrit (%) iii	14.9-36.7	25.5 ± 1.13	26.9-43.5	34 ± 0.99	< 0.001
MCV (fl) iv	49-77.4	64.4 ± 1.78	48.6-75.8	62.3 ± 1.56	0.416
MCH (pg) v	14.2-25.3	20.26 ± 0.73	13.4-22.4	18.85 ± 0.49	0.153
MCHC (g/dl) vi	27.3-34	31.36 ± 0.58	27.7-32.4	30.28 ± 0.31	0.077
RDW (%) vii	14.2-42.4	21.33 ± 1.49	13.4-24.3	18.92 ± 0.38	0.095
WBCs count (*109/l) viii	2.8-13.4	7.24 ± 0.786	5.4-14	7.93 ± 0.449	0.064
Platelet count (*109/l) ix	127-524	297.8 ± 18.15	183-465	320.72± 26.33	0.467
MPV (fl) x	8.1-10.5	9.35 ± 0.35	8.5-11.2	10.95 ± 0.25	0.228

i Normal 4.5-5.5 $*10^{12}$/l for males and 3.8-4.8 $*10^{12}$/l for females [5]

ii Normal ≥ 13 g/dl for males and ≥ 12 g/dl for females [5].

iii Normal ≥ 40 % for males and ≥ 36 % for females [5].
iv Normal 76-98 fl [5].
v Normal 27-32 pg [5].

Table 3: Relation between Hb concentrations and other red cell parameters, WBC and platelet count in iron deficiency anemia:

Parameter	Mean ± SE	Hb concentration (g/dl) Mean ± SE	P-value
Hct (%)	25.5 ± 1.13	8.29 ± 0.43	< 0.001
RBC count (* 1012/l)	4.08 ± 0.13		< 0.001
MCV (fl)	64.4 ± 1.78		< 0.001
MCH (pg)	20.26 ± 0.73		< 0.001
MCHC (g/dl)	31.36 ± 0.58		< 0.001
RDW (%)	21.33 ± 1.49		< 0.001
WBC count (*109/l)	7.24 ± 0.786		< 0.001
Platelet count (*109/l)	297.8 ± 18.15		< 0.001

Table 4: Relation between Hb concentrations and other red cell parameters, WBC and platelet count in β–thalassemia trait:

Parameter	Mean ± SE	Hb concentration (g/dl) Mean ± SE	P-value
Hct (%)	34 ± 0.99	10.31 ± 0.3	< 0.001
RBC count (* 1012/l)	5.47 ± 0.07		< 0.001
MCV (fl)	62.3 ± 1.56		< 0.001
MCH (pg)	18.85 ± 0.49		< 0.001
MCHC (g/dl)	30.28 ± 0.31		< 0.001
RDW (%)	18.92 ± 0.38		< 0.001
WBC count (*109/l)	7.93 ± 0.449		< 0.001
Platelet count (*109/l)	320.72± 26.33		< 0.001

Table 5: Relation between Hb concentrations multiplied by 3 and Hct in iron deficiency anemia and β–thalassemia trait:

Type of hypochromic anemia	Hb concentration (g/dl) *3 Mean ± SE	Hct % Mean ± SE	Hb concentration (*3) Hct % Mean ± SE	P-value
Iron deficiency	24.24 ± 0.91	25.93 ± 0.86	-1.68 ± 0.04	0.1838
β–thalassemia trait	30.92 ± 0.9	34 ± 0.99	-3.14 ± 0.07	0.0236

Discussion:

The main drive for this study was the observation that many hospitals and health-care centers are neither having a hematologist nor specific diagnostic tests for the commonest types of anemias including iron deficiency and β–thalassemia trait, however, a good proportion of these hospitals and centers are either already having a hematology autoanalysers or are planning to introduce one in the near future. Therefore, it seems logical to discuss the results that they should face in at least some of the commonly encountered situations in practice.

The hematology autoanalyser per se is considered as the ideal screening test for hypochromic anemias and the explanation of the many parameters generated by the machine can be very helpful [6].

In this study, the sex ratio is referring to that the female patients are having more iron deficiency anemia and this is an acceptable fact [5], yet the sex ratio is also in favor of the females even in β–thalassemia trait, and this may be attributed to the physicians tendency to investigate more for the presence of anemia in females (table 1).

The age distribution reveals that more patients are diagnosed at younger age (table 1).

The red cells count can be described as being mostly in the lower normal threshold or even below it in iron deficiency anemia, while it is in the upper normal threshold or even above it in β–thalassemia trait. Also there is a statistically highly significant difference in red cells count between the two types of anemia (table 2).

There is also a highly significant difference in Hb concentration and Hct percent between iron deficiency anemia and β–thalassemia trait, with the more severe anemia associated with iron deficiency and the mild anemia or even having Hb concentration and Hct percent in the lower normal values associated more with β–thalassemia trait (table 2).

All patients with lower normal Hb concentration and Hct percent and presence of clear hypochromia and microcytosis are β–thalassemia trait (table 2).

Surprisingly, the difference in RDW percent is not statistically significant, although there is some apparent difference between the two conditions. However, RDW higher than 24.3 % is only associated with iron deficiency anemia (table 2).

All other parameters including MCV, MCH, MCHC, WBCs count, platelets count and MPV are not useful to differentiate between the two types of anemia (table 2).

There is a highly significant relation between all red cell parameters and the level of anemia represented by Hb concentration in both iron deficiency anemia (table 3) and β–thalassemia trait (table 4). Also there is a similarly highly significant relation between the WBC and platelets count and Hb concentration in both types of anemia (tables 3 and 4). This refers to that the hypochromic anemia itself and not its subtype or specific cause is linked to WBC and platelets count.

In normal subjects the Hct percent is usually three times the Hb concentration [5], and although this relation should not be applied as a rule in practice, but still it is helpful to demonstrate the general thinking in the relation between the two parameters. This study demonstrated that relying on Hb concentration rather than on Hct percent to evaluate the presence of anemia makes its diagnosis more obvious. There is a statistically significant difference between Hb concentration and Hct percent in β–thalassemia trait in which the Hct percent is higher than the corresponding hemoglobin concentration. A similar provisional finding could be noted in iron deficiency anemia, but it is not significant statistically, although still the level of Hb concentration is slightly below Hct percent in most of the cases (table 5).

Finally, the ideal environment for the diagnosis and classification of hypochromic anemia would include, beside the specialized hematologist for evaluation of the blood smear, a hematology autoanalyser and all the confirmatory tests for the subtype of anemia. However, even in the less than ideal environment the provisional diagnosis of the subtype of anemia would give a very useful lead for the clinician.

Conclusions:

1-Red blood cells count is mostly in the lower normal value or below in iron deficiency anemia, and it is in the upper normal value or above in β–thalassemia trait.

2-β–thalassemia trait usually presents with milder anemia or even with lower normal Hb concentration and Hct percent. While in iron deficiency anemia the anemia is always present and it tends to appear more severe than in β–thalassemia trait.

3-Relying on Hb concentration rather than on Hct percent to evaluate the presence of anemia makes the diagnosis more obvious.

4-RDW higher than 24.3 % is only associated with iron deficiency anemia.

5-MCV, MCH, MCHC, WBCs count, platelets count and MPV are not useful to differentiate between iron deficiency anemia and β–thalassemia trait.

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