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Comparison of hematological parameters with peripheral smear in anemia

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Abstract
Background: Being one of the most common disorder faced by our society, the correct diagnosis of anemia is important for better clinical outcome.
Aim & Objectives: The present study was designed to find out the association between cell counter generated hematological parameters and peripheral smear findings in the diagnosis of anemia.
Material and Methods: This study was carried out in a tertiary care hospital in South India. 110 patients with low Hb level with reference to age and sex were selected for the comparison of PS findings with that of cell counter parameters.
Results: Among 110 cases 56 (50.9%) were females and 54 (49.1%) were males and the common age group affected is elderly >60 years (33 out of 110 cases, 30%) followed by patients in 4th and 5th decades (32 out of 110, 29%).Milder forms of anemia was seen to be common in females, while severe anemia was seen more in males. The most common morphological type of anemia was normocytic normochromic anemia (57%) followed by microcytic hypochromic anemia (44%). Among the 57% of normocytic normochromic anemia cases, 41(72%) cases were males and among the 44% of microcytic hypochromic anemia, 36 (82%) cases were females. There were few discrepancies between the diagnosis anemias on peripheral smear and cell counter generated parameters. 41 cases of Microcytic hypochromic anemia had high RDW which is normally expected but 29 cases of Normocytic normochromic anemia showed raised RDW. However the mean RDW value among the cases of microcytic hypochromic anemia and normocytic normochromic anemia with raised RDW was 17.7% and 14.1% respectively.
Conclusion: Despite all the advances in laboratory sciences, peripheral smear examination remains an important diagnostic tool in diagnosis of anemia.

Keywords: Anemia, peripheral smear examination, RBC indices

Introduction

Anemia is defined as decrease in the total number of red blood cells or Hemoglobin in the blood or lowered ability to carry oxygen. It is one of the most common problems faced by our society which is often ignored worldwide both in developed and developing countries. It is a condition that occurs when the red blood cells do not carry enough oxygen to the tissues of the body. Preschool children, pregnant women and adolescents constitute vulnerable group of anemia [1]. From being the most common nutritional deficiency disorder in the world, other causes of anemia like hemolysis are also common [2]. Therefore correct diagnosis of type of anemia is very important for the right therapy to be administered. It is not a diagnosis itself, but it is a sign of underlying disease. In the diagnosis of different anemia, morphological changes of red blood cells provide an important diagnostic value.

Peripheral smear (PS) examination is one of the basic and informative tools for screening, diagnosis and monitoring of type of anemias and to look for therapeutic response [3]. Anemia is better classified into its types on the basis of both qualitative and quantitative results. An initial morphologic classification of anemia with integration of red blood cell indices and morphologic characteristics is probably most useful. Hence it is categorized by cell size as microcytic, normocytic, or macrocytic. Microcytic anemia associated with iron deficiency is the most prevalent micronutrient deficiency disease in the world affecting 2 billion people [4]. Over the past few years, complete blood count (CBC) by the automated hematology analyzers and microscopic examination of peripheral smear have complemented each other to provide a comprehensive report on patient's blood sample.

Automated hematology cell counters have improved accuracy and precision, has reduced subjective errors and safety in handling of blood specimen [5]. The benefit of manual scan is to detect clinically significant morphological abnormalities (pencil cells, sickle cells, tear drop cells, schistocytes etc.) that are not quantifiable by the instrument. Therefore the results of hematology analyzer should validate with manual scan of peripheral blood smear and both the results must correlate with each other [6].

Aims and Objectives

- To find out the association between hematological parameters and type of anemia.

Materials and Methods

The present study was carried out in a tertiary care hospital for a period of two months.

The present study was a cross sectional study. The study samples were both inpatient and outpatient from all departments of all age groups. 110 patients with hemoglobin concentration for the diagnosis of anemia according to World Health Organization were selected. Two ml sample of the patients collected in Ethylene diamine tetra acetic acid (EDTA) bulb, was processed for CBC in Beckman Coulter and Peripheral Smear was prepared from the same sample.

The cell counter generated parameters which included red blood cell (RBC) indices (MCV, MCHC, MCH), RDW (Red cell distribution width) and platelet counts were analyzed. The peripheral smears prepared were stained by standard protocol with Leishman stain. After preparation, each peripheral smear was examined for the morphological abnormalities associated with different types of anemia and results were noted. The diagnosis obtained by both the methods i.e. PS examination and cell counter generated parameters were correlated.

Reference Range

Table 1: Assessment of severity of anemia according to World Health Organization [7, 8].

Age	Mild	Moderate	Severe
6 – 59 months	10 – 10.9	7 – 9.9	<7
5 – 11 years	11 – 11.4	8 – 10.9	<8
12 – 14 years	11 – 11.9	8 – 10.9	<8
Female >14 years	11 – 11.9	8 – 10.9	<8
Male > 14 years	11 – 12.9	8 – 10.9	<8

Mean Cell Volume (MCV) – 76 – 96 fL
Mean Cell Hemoglobin (MCH) – 27 – 32 pg
Mean Cell Hemoglobin Concentration (MCHC) – 32 – 36 g/dl
Red cell distribution Width (RDW) – 11.5 – 13.5 %

Results

The following results were obtained. Among 110 cases 56 (50.9%)were females and 54 (49.1%)were males. Severe anemia was most common in our study followed by moderate and mild. There was not much statistical difference in moderate and severe anemias. Milder forms of anemia was seen to be common in females, while severe anemia was seen more in males.

Table 2: Age wise distribution and grading of anemia

	<13	13 - 18	19 - 30	31 - 40	41 - 50	51 - 60	>60	Total
Mild	1	1	3	0	2	3	4	14
Moderate	6	5	4	3	14	2	13	47
Severe	1	3	4	3	16	6	16	49
	8	9	11	6	32	11	33	110

Anemia more common in our study was age group more than 60 years (33 out of 110 cases, 30%) followed by between 41 to 50 years (32 out of 110, 29%).

Table 3: Gender wise distribution of type of anemia

	Male	Female	Total
Microcytic hypochromic	8	36	44
Normocytic normochromic	41	16	57
Macrocytic	1	1	2
Dimorphic	4	2	6
Hemolytic anemia	0	1	1
	54	56	110

In males normocytic normochromic anemia was more common (41 out of 57 cases, 72%) and in females microcytic hypochromic anemia was more common (36 out of 44 cases, 81%).

Table 4: Mean Corpuscular Volume (MCV) vs distribution of type of anemia

	Normal	Low	High	Total
Microcytic hypochromic	6	37	1	44
Normocytic normochromic	49	3	5	57
Macrocytic	0	0	2	2
Dimorphic	5	1	0	6
Hemolytic anemia	1	0	0	1
	61	41	8	110

Out of 44 cases of microcytic hypochromic anemia 37(84%) cases showed low MCV but 6 (14%) cases showed normal MCV. When compared, the anemia diagnosed based on MCV values and by manual examination there was a significant difference.

Out of 57 cases of normocytic normochromic anemia, 49(86%) cases showed normal MCV level.

Table 5: Red cell Distribution Width (RDW) vs distribution of type of anemia

	Normal	Low	High	Total
Microcytic hypochromic	2	1	41	44
Normocytic normochromic	19	9	29	57
Macrocytic	0	0	2	2
Dimorphic	0	0	6	6
Hemolytic anemia	0	0	1	1
	21	10	79	110

41 cases of Microcytic hypochromic anemia had high RDW which is normally expected but 29 cases of Normocytic normochromic anemia showed raised RDW. The mean RDW value among the cases of microcytic hypochromic anemia and normocytic normochromic anemia with raised RDW was 17.7% and 14.1% respectively.

Normocytic normochromic anemia was associated with more severe forms of anemia, followed by microcytic hypochromic anemia. In our study it was also found that Platelet counts were higher in microcytic hypochromic anemia (14 of 44 cases, 31.82%).

Discussion

The peripheral blood smears in anemia were evaluated and it was compared with cell counter generated red cell indices of 110 patients. In our study 49.1% were males and 50.9% were females, which was similar to Singla *et al.* [9] in which 53.6% were females and 46.4% were males in contrast to Khan *et al.* [10] in which 52.63% were males and 47.36% were females.

44.5% of the cases showed severe anemia followed by moderate in 42.8% and mild in 12.7% cases. In our study it was found that milder forms of anemia was seen in females and more severe forms seen in males which was in contrast to the study done by Khan *et al.* [8] in which mild anemia was common in males and severe anemia in females.

Majority of the patients in our study were elderly (33%) followed by adults (32%) in the age group of 41 to 50 years. Study done by Kaur *et al.* [11] in which 55% were found in the age group of 60 – 69 but the study done by Singla *et al.* [9] showed 21% of the cases were within 21 to 30 years.

Multiple conditions can lead to anemia in adults and elderly persons, which includes chronic disease and inflammation, chronic kidney disease, decreased marrow response, along with decreased ratio of bone marrow to fat cells.[12]EPO regulation occurs primarily in kidney with a smaller contribution by liver hepatocytes and this is a major cause of normocytic normochromic anemia [13].

The most common morphological type of anemia was normocytic normochromic anemia (57%) followed by microcytic hypochromic anemia (44%) and dimorphic anemia in 6% cases similar to the study by Kaur *et al.* [11] (56% of normocytic normochromic anemia and 34% of Microcytic hypochromic anemia). This finding was in contrast to Khan *et al.* [10] and Patel *et al.* [14] in which microcytic hypochromic anemia was the predominant type, 49.10% and 72% respectively.

Alvarez-Uria G *et al.* [15] in their study found that microcytic anemia was more prevalent in children and women, the proportion of normocytic anemia increased progressively with age in male adults and women after menopause age. This is comparable to the results of the present study.

Among the 57% of normocytic normochromic anemia cases, 41(72%) cases were males and among the 44% of microcytic hypochromic anemia, 36 (82%) cases were females. This can be explained by the fact that normocytic normochromic anemia can be due to chronic disease and inflammation, chronic kidney disease, decreased marrow response. Since our hospital has a renal transplantation unit, most of our cases were anemia of chronic kidney disease which in turn causes reduced erythropoietin production [12]. In females the body needs more iron when it grows rapidly and when frequent blood loss occurs (e.g. menstruation), thus, many women in their reproductive age group are at high risk of developing iron deficiency anemia [9].

Out of 110 cases, 61(55.5%) cases showed normal MCV, 41(37.3%) with Low MCV and 8(7.3%) with high MCV values. Singla *et al.* [9] in their study found 49. 2%) cases showed low MVC, 44.4% cases showed normal MCV and 6.4% showed high MCV values. In our study 37(84%) out of 44 cases of microcytic hypochromic anemia showed low MCV values and 49(86%) out of 57 cases of normocytic normochromic anemia showed normal MCV values which was statistically significant with p value <0.01. But 6 (14%) cases of microcytic hypochromic anemia showed normal MCV and 5 cases of normocytic normochromic anemia showed high MCV values and those cases had mild variation from their normal reference ranges.

41 of 44 cases (93%) of microcytic hypochromic anemia had high RDW which is normally expected but 29 of 57 cases (51%) of normocytic normochromic anemia showed raised RDW. This discrepancy may result in misclassification for the diagnosis of anemia. However the mean RDW value among the cases of microcytic hypochromic anemia with raised RDW was 17.7% but the mean RDW in cases with normocytic normochromic anemia was 14.1%. There was a significant difference in both values. In such cases peripheral smear examination helps to identify the morphology of cells.

Despite improving capabilities of automated hematology analyzers, manual slide review is still necessary to identify some morphologic abnormalities that may be relatively unremarkable in automated methods [16]. It is recognized that the automated systems are superior for counting of red blood cells, HB, MCV, MCH and RDW. Whereas, visual microscopy is superior for differentiating cells. This will ensure proper and appropriate treatment and patient care.[17]Our study found that the microscopic examination of peripheral blood smear is gold standard in the diagnosis of various types of anemia. Similarly Farah *et al.* [18] and Lantis *et al.* [19] in their study also found that the automated hematology analyzers were appropriate for screening purposes because it increases the turnaround time and reduces the labor cost. But to diagnose and differentiate different types of anemia manual scan by peripheral smear was suggested as the best method of choice.

In microcytic hypochromic anemia (14 of 44 cases, 31.82%) platelet counts was high. One of the common causes for reactive thrombocytosis is iron deficiency anemia. The mechanisms causing this are unknown. There are several reports to explain the mechanisms of reactive thrombocytosis from the aspect of thrombopoietin cytokines. Akan *et al.* [20] assayed the serum levels of thrombopoietin, erythropoietin, IL6 and IL11, but none of these cytokines had any effect on reactive thrombocytosis in iron deficiency anemia. Studies done by Bilac *et al.* [21] reported that the amino acid sequence homologous of thrombopoietin and erythropoietin may explain the thrombocytosis. In contrast Recke *et al.* [22] suggested negative participation of thrombopoietin and erythropoietin.

Conclusion

Our study showed the high prevalence of normocytic normochromic anemia (57%) followed by microcytic hypochromic anemia (44%) which differ in various age groups and gender reflecting the varying etiologies behind this. Among the 57% of normocytic normochromic anemia cases, 41(72%) cases were males and among the 44% of microcytic hypochromic anemia, 36 (82%) cases were females. In adults and elderly, most prevalent is normocytic normochromic anemia which may be cause of chronic kidney disease, inflammation, depletion of bone marrow

response and cellularity or ageing process. Platelet counts was elevated in microcytic hypochromic anemia but the underlying mechanism was not known. To clarify the etiology further investigations will be necessary. In the present study anemia is assessed by the results of both hematology analyzer and peripheral blood smear. The results of our study showed that automated systems are superior for hematological parameter analysis but visual microscopy is superior for differentiating cells. The benefit of manual scan is the ability to identify clinically significant morphology of cells that are not quantifiable by instruments. Thus peripheral smear examination is considered as gold standard in the diagnosis of type of anemia.

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