Original Article

Comparative study of anemia cases based on peripheral blood smears and cell counter generated red cell indices

Shruti Singla[1*], Sanjay Bedi[2], Kusum Joshi[3]

[1]Jr. Resident, [2]Professor, Professor and HOD, Department of Pathology, MMIMSR, Mullana, Ambala, Haryana, INDIA.
Email: raghav.garg90@yahoo.com

Abstract

Background: Analyzing peripheral blood smears routinely has facilitated interpretation of various hematological disorders and has been a major diagnostic tool. The advent of automated hematology cell counter has improved accuracy, precision and safety. There is still a need to depend on manual techniques for primary calibration. This highlights the importance of maintaining the manual technical skills, Thus, the present study was undertaken to compare anemia cases based on peripheral blood smears and cell counter generated Red blood cell (RBC) Indices. **Material and Methods:** The peripheral blood smears in anemia were evaluated and compared it with cell counter generated red cell indices of 500 anemic patients. The automated analyzer SYSMEX XP-100 was used.Simultaneously, a peripheral smear was prepared according to standard operating procedures and stained by Leishman stain. **Results:** The cases consisted of normocytic normochromic anemia (14%), microcytic hypochromic anemia (76.2%), macrocytic anemia (0.4%) and dimorphic anemia (13.4%). In normocytic normochromic anemia on peripheral smears 65.7% showed normal curve. In microcytic hypochromic anemia 81.1% showed left shift. In cases of macrocytic anemia 100% histogram showed right shift. Majority of the curves in dimorphic anemia showed broad based curve (46.26%). **Discussion:** The relationship between histogram patterns and peripheral smear diagnosis in dimorphic anemia posed queries regarding the validity of histocytograms. Hence, peripheral smear examination along with clinical history is an important diagnostic tool while handling the patients with hematological conditions.

Key Words: Anemia, peripheral smear, automation, red cell indices.

[*]**Address for Correspondence:**
Dr. Shruti Singla, Jr. Resident, Department of Pathology, MMIMSR, Mullana, Ambala, Haryana, INDIA.
Email: raghav.garg90@yahoo.com

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INTRODUCTION

Since decades, peripheral blood smear has been used as window to observe hematological ongoings. Analyzing peripheral blood smears routinely has facilitated interpretation of various hematological disorders and has been a major diagnostic tool[1]. Cell counters have penetrated medical laboratory services in a ubiquitous manner with increasing efficacy and decreasing cost all over the world. Over the past few years, complete blood count (CBC) by the automated haematology analysers and microscopic examination of peripheral smear have complemented each other to provide a comprehensive report on patient's blood sample[2-4]. The advent of automated hematology cell counter has improved accuracy and precision, has reduced subjective errors and safety in handling of blood specimen. There is still a need to depend on manual techniques for primary calibration despite, the sophistication of present day instruments. This highlights the importance of maintaining the manual technical skills, and to ensure this by appropriate technician training program, despite the temptation to leave it all to the machines. Thus, the present study was undertaken to compare anemia cases based on peripheral blood smears and cell counter generated Red blood cell (RBC) Indices.

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MATERIAL AND METHODS

The present study was undertaken in the central diagnostic laboratory of a tertiary care hospital. A total of 500 patients with anemia, defined as per World Health Organization (WHO) criteria, were studied over a period of two years from September 2014 to July 2016. A 3ml of EDTA venous blood sample was collected from the patient and a histogram was obtained after thorough mixing of the sample. The automated analyzer SYSMEX XP-100 is a three part differential automated analyzers which was used for the study. Simultaneously, a peripheral smear was prepared according to standard operating procedures and stained by Leishman stain. This peripheral smear was reported by pathologist who was not privy to histogram during the reporting of peripheral smear. A qualitative analysis of the data was done using Pearsons Chi square test and Fisher exact test wherever appropriate.

RESULTS

The age group of patients included in this study ranged from an infant aged 1 year to 85 years. Majority of patients (21. 0%) were within 21-30 years of age. Out of total 500 patients, 53.6% were females and 46.4% were males (Table 1).

Table 1: Age and Gender Wise Distribution

		Sex		Total
		Male	Female	
Age	1-10	32	31	63
	11-20	26	34	60
	21-30	31	74	105
	31-40	27	47	74
	41-50	45	25	70
	51-60	31	17	48
	61-70	26	22	48
	71 and Above	14	18	32
	Total	**232**	**268**	**500**

Chi Square=32. 30; df =7; p<0. 05

According to the hemoglobin values, anemia was divided into mild (Hb <11 gms), moderate (Hb 7-10gm%) and severe (Hb <7 gm%) anemia. Out of the 500 patients, majority cases 239 (56%) showed moderate anemia, 124 (29%) showed severe and 64 (15%) showed mild anemia. In present study, 246 (49.2%) cases had MCV less than 80 fl, 222 (44.4%) cases were showing normal MCV and 32 (6.4%) cases had MCV >100 fl. When compared the anemia diagnosed based on MCV values and by manual examination of peripheral smear there was a significant difference. It was mainly due to dimorphic anemia. Dimorphic anemia cases were included in the normal range of MCV i.e., 80-100fl. A total of 264 (52.8%) cases had MCH less than 26 fl, 210 (42%) cases had normal MCH and 26 (5.2%) cases had MCH >34pg. Whereas, 185 (37%) cases had MCHC less than 31gm/dl, 304 (60. 8%) cases were showing normal MCH and 11 (2.2%) cases showed MCHC >37gm/dl. Majority of cases were in normal range. On analyzing the data it was found that, majority(71.4%) of blood smears which were reported as normocytic normochromic anemia on manual examination showed MCH ranging from 26-34pg. In case of microcytic hypochromic blood picture majority (60.9%) showed MCH <26pg. In our study, we had two cases of macrocytic anemia. Both the cases (100%) showed MCH > 34pg. In cases which were reported as Dimorphic anemia there was no specific range for MCH. When compared the anemia diagnosed based on MCH values and by manual examination of peripheral smear there was very high significant difference with p value of <0.01. The significant difference was mainly cause of dimorphic anemia. Dimorphic anemia cases were included in the normal range of MCH i.e., 26-34pg. Out of 500 cases, 404 cases (80.8%) cases showed RDW-CV values more than 19.2% which correlated with the cases of microcytic anemia 11.6% showing Broad Base curve. RDW –CV >14.6 are considered as microcytic anemia which in this study correlated with the peripheral smear findings. Out of 361 cases of microcytic hypochromic anemia diagnosed manually, 83.1% (300) cases showed RDW > 14.6. Histogram pattern showed Left Shift in 341 (68.2%) cases, Normal Curve in 19%, Bimodal Curve in 0.8% and Right Shift in 0.4%.

Table 2: Correlation of Peripheral Smear findings and Histogram patterns

		Histogram					Total
		NC	LS	RS	BB	BM	
PS	NN	46	22	1	1	0	70
	MH	43	293	0	25	0	361
	MA	0	0	2	0	0	2
	DA	6	26	0	31	4	67
Total		**95**	**341**	**2**	**58**	**4**	**500**

p < 0.001(very highly significant), Fishers exact test =118. 45; df =12

(PS= Peripheral smear; NN= Normocytic normochromic; MH= Microcytic hypochromic; MA= Macrocytic anemia; DA= Dimorphic anemia; NC= Normal curve; LS= Left shift; RS= Right shift; BM= Bimodal curve) Most of the findings on peripheral smears correlated well with the histogram patterns. The very high significant difference when we compare the anemia diagnosed by histograms and by manual examination of peripheral smear. The histogram pattern correlated with majority cases of microcytic hypochromic, normocytic normochromic and macrocytic anemia. However, variations of histogram patterns were seen in dimorphic anemia.

DISCUSSION

The peripheral blood smears in anemia were evaluated and compared it with cell counter generated red cell indices of 500 anemic patients. In our study, it was seen that out of 500 cases, majority of cases fall in the adult age group of 21 to 49 years and among 268 female patients majority (70.8%) fall in reproductive age group. These results were in concordance with the studies conducted by Kumar *et al*[5], Cook *et al*[6] and Japheth *et al*[7]. This can be explained as the period of adolescence and adult group is a period of intense growth and development and iron is in high demand as it is present in all body cells and is fundamental for basic physiological processes such as Hemoglobin formation. The body needs more iron when it grows rapidly and when frequent blood loss occurs (e. g. menstruation) thus women in reproductive age group are at high risk of developing iron deficiency anemia. After the age of 40 years, males were seen to be more affected than females. In our study we considered all cases with hemoglobin less than or equal to 11gms%. The mean hemoglobin was 9.24gm%. Majority 50% of cases had hemoglobin 7-10gm/dl.Kumar *et al*[5] and Patel *et al*[8] studies also showed mean hemoglobin as 7.2 gm% and 5.85gm% respectively. The most common morphological type of anemia was microcytic hypochromic anemia (76.4%) followed by normocytic normochromic anemia. Iron deficiency anemia is the most common cause of microcytic hypochromic blood picture. WHO has estimated that prevalence of anemia in pregnant women is 14% in developed and 51% in developing countries and 65-75% in India[9]. About one third of the global population (over 2 billion) are anemic. Prevalence of anemia in all the groups is higher in India as compared to other developing countries[10]. Out of 500 cases, 246(49. 2%) cases showed MVC less than 80fl, 44.4% cases showed normal MCV and 6.4% showed MCV more than 100fl. Majority of anemia cases showed normal MCV. This was in concordance with other studies[8,11]. In a study conducted by Patel *et al* in 2009, majority (72%) of the patients with anemia showed MCH <26pg, 22% showing normal MCH and only 6% showing MCH >34pg. Both the studies show majority of patients with anemia showing MCH <26pg[8]. Very few studies have been conducted on the utility of red cell histograms in identifying common hematological disorders[12]. With most of the studies favoring white cell histograms and their use in identifying and characterizing leukemia blast populations[13]. The RBC histogram is an integral part of automated hematology analysis and is available routinely on all automated cell counters. The histogram in association with other CBC parameters such as RBC distribution width and mean corpuscular volume has been found abnormal in various hematological conditions[12,14,15]. In the present study 500 patients of anemia were analyzed and we compared their peripheral smear report with the Red blood cell histogram pattern obtained from SYSMEX XP-100 is a three parts differential automated analyzer. In view of maximizing the usefulness of the histogram a dotted line depicting a reference normal curve was drawn super imposed on every red cell histogram so any discernible deviation from that curve can be clearly delineated for contrast. We noticed that in smears reported as microcytic hypochromic anemia 81.1% histograms showed left shift, 65% of normocytic normochromic smears showed normal curve and all the smears having macrocytic blood picture showed right shift pattern of histogram. Thus we can see that histograms are useful diagnostic aid when it comes to normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic anemia. However, the dimorphic anemia showed different histogram patterns from simple curve to complex curves. In the smears reported as dimorphic anemia we noticed that only 5.9% of histograms showed bimodal curve, whereas majority 46.26% showed broad base histogram pattern and 38.8% showed left shift histogram curve. The broad base curve can be explained by the presence of multiple populations of cells of varying sizes (i.e., normocytic, microcytic and macrocytic). Our study was in concordance with the study conducted by Constantino *et al* in 2010. The significant difference was largely due to dimorphic anemia cases which was in concordance with Constantino *et al*[16]. The bimodal red cell histograms are usually associated with therapeutic transfusions and/or hematinic agent response to microcytic and macrocytic anemia, but they may also indicate other hematological disorders such as early iron developing microcytic population, folate/vitamin B12 developing macrocytic population, post-iron treatment of iron deficiency anemia and post-iron treatment of iron deficiency with megaloblastic anemia, etc. In Dimorphic anemia the histogram pattern, the centeredness and the width shows the variations in the RBCs. The dimorphic blood picture will look like a dual population of microcytic and normocytic or normocytic and macrocytic red cells or a admixture of small, normal and large cells of different sizes and forms with or without normal red blood cell indices which can mislead the diagnosis if we rely on automated values alone, thus it is important to examine the peripheral blood smear to examine all the populations of the cell. Practically since dimorphic is usually associated with abnormal red cell populations, morphological findings should be correlated with the graphical and numerical data for better interpretation of results. In the present study, we also classified 500 cases of anemia based on the red cell indices (MCH, MCV and

MCHC) obtained from lab life D5 supreme automated 5 part analyzer. These findings were compared with visual peripheral smear examination as the gold standard. MCV cases with less than 80 fl were considered microcytic, 80-100 were considered normocytic or dimorphic and more than 100 fl were considered macrocytic. MCH less than 26 were considered microcytic,26-34 pg were considered normocytic, normochromic or dimorphic and more than 34 pg were considered as macrocytic anemia. On applying statistical analysis to the two variables i.e., anemia classified with peripheral smear and anemia classified by red blood cell indices we noticed the P value showing significant difference. In study done by Bowen *et al*, 61% of anemic patients had MCV in normal range i.e., 80-100fl[13]. This was partly in correlation with our study which showed normal MCV in 44.4% (222). Hence it can be concluded that MCV is an insensitive marker to classify anemia. The probable reasons as discussed by Aslina*et al* are MCV is an average value and doesn't reflect the presence of different red cell population[18]. Therefore, in all patients with decreased hemoglobin; peripheral smear examination is a must for identifying early red blood cell changes. MCV represents only mean of distribution curve and is insensitive to small number of macrocytes and microcytes. MCH and MCHC gives little information independent of MCV. Bain BJ in her review on the place of peripheral blood smear examination in the age of automation in 2005 stated that even in the age of molecular analysis, the blood smear remains an important diagnostic tool and sophisticated modern investigations of hematologic disorders should be interpreted in the light of peripheral – blood features as well as the clinical context[19]. Visual examination of red cell size distribution histograms from automated hematology instruments is often more sensitive and objective than examination of a blood smear for detecting the presence of a subpopulation of red cell with distinctly different size. In the present study we noticed that majority of cases with microcytic hypochromic anemia showed high RDW thus confirming that RDW is a very sensitive indicator of anisocytosis RDW also shows a better correlation as an indicator of anisocytosis when the MCV is in the low normal range and anisocytosis is difficult to detect like in cases of developing iron deficiency. The red cell histogram of patients with iron deficiency show left shift but on treatment the histogram widens consequently increasing RDW. In nutritional deficiencies like iron, folate or vitamin B12 there is increase in red cell heterogeneity producing more abnormal cells in peripheral blood. In the present study we have noticed that majority of the histogram curves in dimorphic anemia were broad based. This proves that as we have explained before, in dimorphic anemia there are multiple population of red blood cells (i.e., normocytes, microcytes and macrocytes), due to this high rate of anisocytosis we have obtained majority of broad base curves in dimorphic anemia which again will increase RDW values. This was in concordance with other studies conducted by Rowen *et al* and Park *et al*[20,21]. In these studies, RDW showed marked increase in cases of microcytosis. Constantino *et al*stated that higher the RDW higher the degree of anisocytosis and poikilocytosis. The Red cell histograms from automated hematology analyzer gives us a valuable information regarding the various hematological conditions. Very few studies have focused on RBC histograms while giving more significance to the WBC histogram and to the leukemic blast populations. Our study revealed us an important correlation between RBC histograms and peripheral smear diagnosis in Microcytic Hypochromic, Normocytic Normochromic and Macrocytic Anemia. However the relationship between histogram patterns and peripheral smear diagnosis in dimorphic anemia posed queries regarding the validity of histocytograms. Hence it was concluded that in the age of molecular analysis and automation, peripheral smear examination along with clinical history is an important diagnostic tool while handling the patients with hematological conditions. Red cell histograms along with numerical parameters like MCV, MCH, MCHC and RDW act as an aide to visual examination of peripheral smears.

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