

Comparison of classification of anemia based on mean corpuscular volume by hematology analyzer and peripheral smear examination

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ABSTRACT

Objective: This study was conducted to identify different morphological patterns of anemia based on mean corpuscular volume determined by a hematology analyzer and comparing it with peripheral smear examination.

Material and Methods: A total of 94 anemic patients were studied at Punjab Institute of Cardiology. Anemia was characterized by a decrease in hemoglobin (Hb) concentration below normal limit i.e <12g/dl in women and <13.0 g/dl in men using an automated analyzer. Morphological classification was done based on peripheral smear examination findings and mean corpuscular volume (MCV). SPSS version 26 was used for data analysis. Frequencies were calculated for gender and subtypes of anemia and its severity was calculated into percentages. Age was calculated as mean and SD. Post stratification Chi-square test was applied to compare PSE and automated analyser taking p value of more 0.0001 as significant.

Results: The mean age of included patients was 34.88± 15.25 years with minimum and maximum age 7 months old and 85 years. Females were more commonly affected than males with male to female ratio 1:2. Majority, i.e. 53% of patients suffered from moderate degree of anemia while 39% participants had hypochromic microcytic pattern of anemia. Post stratification Chi-square test was applied to compare peripheral smear examination and automated analyzer which gave a significant p value of 0.0002.

Conclusion: This study emphasizes the role of PSE in comparison with automated hematology analyzer for the diagnosis and subtyping of various forms of anemias.

Keywords: Anemia, Microcytic hypochromic, Normocytic normochromic

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INTRODUCTION

Anemia is functionally defined as an insufficient RBC mass to supply oxygen to peripheral organs. Hematocrit, red blood cell (RBC) count and RBC indices also play an important role in classification of anemia. Proper management of patients relies on identification of anemia and its subtypes [1]. Hemoglobin (Hb) is

reflected by underlying nutritional status. The reduction in the Hb concentration of the peripheral blood below the normal limit or the reduction in hematocrit below the lower limit of the 95% reference interval in relation to age and gender is called anemia. It is an expression or sign of an underlying disorder [3]. By WHO criteria, an Hb of < 13 gm/ dl in males and <12g/dl in females is classified as anemia [4]. However, age and pregnancy have different reference intervals. There are numerous etiologies for different categories of anemia such as nutritional, hemolytic, aplastic, hemorrhagic, sideroblastic anemias, and anemia of chronic disease [8].

Patients suffering from anemia may present with all kinds of different symptoms

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depending upon their underlying disease status such as lethargy, light headedness, fainting, poor appetite, palpitations and poor weight gain [7]. Anemia generally affects elderly patients more commonly than the younger ones, adversely affecting the overall health [5]. In Pakistan, however, younger population especially females suffer from anemia to a greater extent. The most common subtype encountered are nutritional anemias especially iron deficiency and it is regarded as one of the most important factors leading to various health related complications such as obstetrical issues [6]. Nutritional anemias are also directly related to educational status of individual patients. Illiterate or less educated females are more commonly affected than the ones who had basic education or access to schools. Personal habits such as smoking also contributes to anemia [2].

Morphological appearance of RBCs studied on a stained blood smear is the most convenient, cost-effective and quickest way of classification of anemia into normocytic, microcytic and macrocytic anemia by determining the size of RBCs. By visualizing the Hb content of individual RBCs on peripheral smear examination (PSE), hypochromic or normochromic nature of anemia can also be determined. However, the use of peripheral smear does carry a risk of observer bias. Automated hematology analyzer is another tool used for diagnosis of anemias. This study will focus on the comparison of peripheral smear examination with automated hematology analyzer for diagnosis and subtyping of anemia while also focusing on the different morphological patterns of anemias encountered

MATERIAL AND METHODS

A total of 100 anemic patients were studied for morphological pattern of anemia based on red cell indices and PSE. Prior approval was obtained from IEC and informed consent was obtained from all patients who participated in the study. Anemia was graded into mild, moderate and severe according to WHO criteria [3] (Table-I). Patients with anemia, characterized by decrease in hemoglobin concentration below normal limit i.e. <12.0 g/dl in women and <13.0 g/dl in men [3]. Patients with known systemic

illness, hematological disorders and neoplastic disease who had taken radiotherapy or chemotherapy were excluded to avoid selection bias.

By definition, microcytic anemia was defined as mean corpuscular volume (MCV) below 80 fl, MCV between 80 and 100 fl as normocytic and MCV above 100 fl as macrocytic [9]. Mixed deficiency anemia was characterized as normal MCV with raised red cell distribution width (RDW). Whole blood was taken into EDTA vacutainer and analysed using automated cell counter (Mindray BC-6000 cell counter 5 part). Microscopy (Peripheral smear examination) was performed on slides stained with Geimsa stains for categorization of anemia into various morphological subtypes. 2 slides were prepared for each specimen and examined by two different hematologists to minimize chances of observer bias.

Statistical Package for the Social Sciences (SPSS) version 26 was used for entry of data and analysis. Qualitative variables such as gender was calculated into frequencies, while subtypes of anemia and its severity was calculated in to percentages. Continuous variables like age was calculated as mean and SD. Data was stratified for age, gender and morphological subtypes based on microscopy and automated analyser. Post stratification Chi-square test was applied which equalled to 22.404 with p value of 0.0002 which was highly significant.

RESULTS

Amongst 94 patients enrolled in the study, the youngest was seven months old infant and eldest one being 85 years old. The mean age was 34.88 ± 15.25 years. Young patients of 20-29 years were the most affected age group. (Figure-III) Females were more commonly affected than males. There were 63 females and 31 males with male to female ratio 1:2 (Figure-I).

Severity of anemia was categorized into mild, moderate and severe (Figure-II) Morphological typing of anemia was done based on PSE findings. The analysis revealed 39 (39%) participants had hypochromic microcytic pattern, followed by 28 participants (28%) with a

normocytic normochromic pattern, 19 participants had mixed deficiency picture showing both hypochromic microcytic as well as macrocytic pattern of anemia whereas 09 participants (09%) had macrocytic anemia and 5 (5%) patients suffered from hemolytic anemia with presence of schistocytes and nucleated red blood cells (Table-III). The findings of PSE and automated analyzer were compared and post stratification Chi Square test results revealed p-value of 0.0002 which was highly significant.

Table-I: Severity of anemia.

Severity	Hemoglobin concentration (g/dL)
Mild	Men-11-12.9 Women 11-11.9
Moderate	8-10.9
Severe	< 8

Table-II: Distribution of morphological patterns of anemia.

Type of anemia	No of patients (%)		Total
	Male	Female	
Normocytic normochromic anemia	11 (11.7)	16 (17)	27
Hypochromic microcytic anemia	12 (12.7)	24 (25.5)	36
Macrocytic anemia	4 (4.2)	5 (5.3)	09
Mixed deficiency anemia	5 (5.3)	12 (12.7)	17
Hemolytic	1 (1)	4 (4.2)	5
Total	33	61	94

Table-III: Comparison between peripheral smear and auto analyzer interpretation in cases of different morphological anemia.

Type of anemia	Auto analyzer	PBS
Normocytic normochromic (%)	33 (35.1%)	26 (27.6%)
Microcytic hypochromic (%)	49 (52.1%)	37 (39.3%)
Macrocytic (%)	8 (8.5%)	09 (9.5%)
Dimorphic (%)	2 (2.1%)	18 (19.1%)
Hemolytic (%)	01 (01%)	04 (4.2%)
Total	94	94

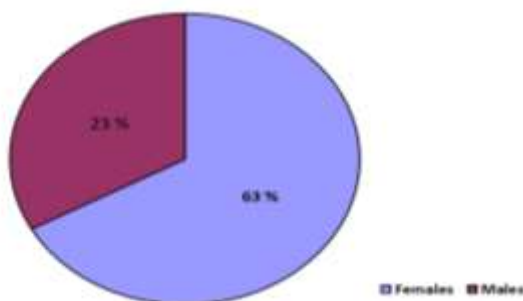


Figure-I: Gender wise distribution of patients.

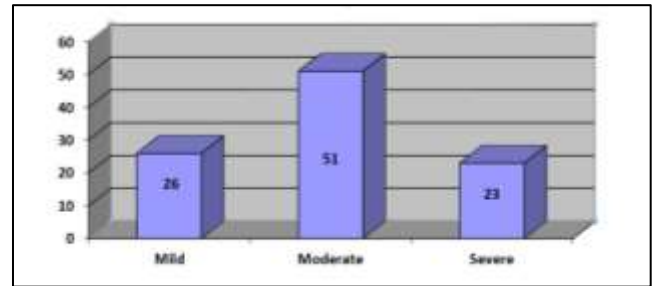


Figure-II: Distribution of patients based on severity of anemia.

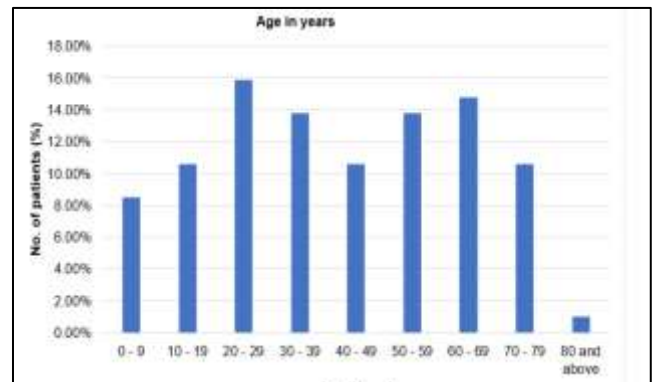


Figure-III: Age distribution of patients.

DISCUSSION

Developing countries like Pakistan face one of the biggest challenges of curtailing anemia. Poverty, lack of awareness and education along with limited access to hospitals with majority of the population living in rural areas are contributory elements in these areas [3]. In this study, patients of both genders were observed which is in contrast to other studies where special groups such as pregnant females and children were targeted. This has given us a valuable insight into various patterns of anemia but also helped in determining that if it is done on a larger scale, can be extremely valuable in defining reference ranges for the same.

Diagnosis of anemia and appropriate management of patients rests on correct categorization of morphological subtype of anemia. It can be done using automated technology which has improved precision, accuracy and reduces the subjective error and is efficient [10]. Peripheral smear is employed as crucial diagnostic technique for anemia, leukemia and other hematological disorders as an adjunct to automated counts. It carries the advantage of being cost-effective and can be carried out in any setup with minimal equipment needs [11].

Anemic patients observed in this study belonged to a wide range of age groups. The youngest patient was a seven months old infant and eldest one was an 85-year-old individual. In another study conducted by Chand FM *et al.* the mean age was 54.36 ± 8.21 years, however this study was conducted on patients suffering from myocardial infarction [13]. The most affected group was 20-29 years old with a female predominance. Increased nutritional needs accompanied with regular blood loss and obstetrical phenomenon can explain the possible relationship of this age group [12]. Male to female ratio in this study was 1:2. This is similar to a study conducted by Solomon D *et al* where male to female ratio of 1:1.745 was present [14].

Microcytic hypochromic anemia was the most common morphological category of anemia in our study, encountered in 39% of the patients. This was in accordance with an observation by Ongole AP *et al* who found 47.8% patients with same morphological subtype in their study and Patel S *et al* whose results showed 46% patients with similar subtype [15]. In a study done by Ashraf FF *et al* the most common pattern of anemia was normocytic normochromic 38% whereas hypochromic microcytic anemia was found in 29% of patients [16].

Most of the patients i.e. 51% suffered from moderate degree of anemia 51% followed by mild and severe anemia i.e. 23% and 26% respectively. It is in contrast to another study conducted by Chaudhry *et al.* which showed prevalence of 39% of mild and moderate anemia [17]. While the significance of PSE in determining the subtype of anemia is an established fact, this study was conducted with the purpose of encouraging the use of PSE in resource limited settings and peripheral healthcare centers where automated equipment is not routinely available. PSE, while being cost-effective and time-saving, when correctly carried out, can aid in proper and timely management of patients. It can be used as a guiding tool to carry out further tests in light of morphological subtype of anemia. Automated analyzer is also an effective and swift tool to obtain complete blood counts in routine practice. While PSE carries the risk of observer bias and directly relates to experience of the examining

physician, these risks are eliminated by the use of automated analyzers.

It was observed that certain hematological findings become evident on PSE only as revealed in comparative analysis where few differences were seen in cases of hemolytic anemia and hypochromic microcytic anemia. Normocytic normochromic anemia was found in 34% cases by automated analyzer whereas PSE revealed 28% cases. Which is explainable by the fact that normochromic, normocytic cells may appear normal looking on peripheral smear making the anemia challenging to diagnose. Moderate difference was seen in microcytic hypochromic anemia. Automated analyzer and PSE examination revealed 52% and 39% cases respectively. Giant platelets or platelet clumps and schistocytes in hemolytic anemia can be counted as microcytes and contribute in difference of result by automated analyzer. The findings of macrocytic anemia were more or less the same in two methods. In cases of dimorphic anemias, 3% cases were diagnosed by automated analyser while results of PSE revealed 19% cases. Dimorphic anemia can be mistyped as macrocytic, microcytic and normocytic on basis of predominant RBC population by automated analyzer. Dual population of RBC in dimorphic anemias are better picked on PSE. Most of these findings are in concordance with similar studies conducted by Garg M *et al* and Chavda J *et al.* [18] BJ Bain reviewed PSE in the age of automation in 2005 and found that the blood smear remains an important tool to diagnose anemia and further added that sophisticated latest investigations of hematologic disorders should be analysed keeping in view the findings of peripheral blood features as well as the clinical background [19].

Our study has highlighted that despite minor differences in the diagnosis of different subtypes of anemias by the two methods, the importance of peripheral smear in delineating the underlying cause of anemia cannot be ignored. When carried correctly and with proper expertise, it is a very useful aid in timely diagnosis and proper management of patients in light of morphological subtype of anemia. We encourage

the use of PSE where automated analyzers are not available for the benefit of patients.

CONCLUSION

This study emphasizes the role of PSE in comparison with automated hematology analyzer for the diagnosis and subtyping of various forms of anemias. While encountering minor differences in few subtypes of anemias, the results of both methods have been comparable with a significant p-value of 0.002. This scientifically proves that PSE carries as much importance as automated analyzer for diagnosis of anemia while being easily available, cost-effective, low maintenance and time-saving. It is encouraged to use both methods simultaneously before classifying an anemia as hematology analyzer may miss findings of mixed deficiency and hemolytic anemias.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Sarah Farrukh: Study concept, literature search, data analysis, questionnaire design, final approval to be published

Qurat Ul Ain Ayaz: Conception / design of work, data interpretation, data analysis, final approval to be published

Farhan Ali Khanzada: Literature search, data interpretation, data collection

Huma Sheikh: Data analysis, data interpretation, Drafting

Ambreen Anwar: Literature search, data collection, data interpretation

Soubia Cheema: Literature search, Study concept, questionnaire design

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