Comparison of peripheral blood smear examination with automated haematology analyzer for diagnosing different types of anemia.

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ABSTRACT… Objective: This study aims to determine diagnostic accuracy of peripheral blood smear and automated haematology analyzer and to determine frequency of different types of anemia diagnosed by peripheral blood smear and automated hematology analyzer. Study Design: Cross Sectional study. Setting: Department of Pathology, Rawal Institute of Health Sciences, Islamabad. Period: November 2015 to April 2016. Material & Methods: Sample size of 149 suspected anemia patients was calculated using WHO calculator with 95% confidence interval. Diagnostic accuracy and frequency of anemia types was measured. Chi-square and fissure exact test and ROC curve analysis was applied and significant ($p<0.05$) results were reported. Results: Total 149 patients were included in study. There were 42(28.2%) male and 107(71.8%) female. Mean age of patients was 35.1±2.1SD. Peripheral blood smear and automated haematology analyzer showed sensitivity (68% vs 92%), specificity (59% vs 88%), PPV (72% vs 92%), NPV (55% vs 88%) and diagnostic accuracy (64% vs 91) respectively. Most common type of anemia diagnosed with peripheral blood smear was microcytic hypochromic anemia with raised RDW 36.7% followed by normocytic normochromic anemia with raised RDW 13.3% and macrocytic anemia ($p=0.001$) while in automated haematology analyzer microc ytic hypochromic anemia with raised RDW54.4% followed by normochromic normocytic anemoia with normal RDW 11.1% ($p=0.000$). Conclusion: Automated haematyology analyzer had high diagnostic accuracy for diagnosis of anemia. Microcytic hypochromic anemia and normocytic normochromic are most common anemias diagnosed by peripheral blood smear and automated hematology analyzer and peripheral blood smear cannot be completely replaced by automated haemolytic analyzer. However, if both methods are used simultaneously, more accurate results can be obtained.

Key words: Anemia, Automated Haematology Analyzer, Peripheral Blood Smear.

INTRODUCTION

Anemia is a common hematological disorder affecting one third of population, globally.[1] It is a common condition associated with significant increase in mortality, morbidity, work productivity reduction and neurological development impairment.[2] Anemia is defined as decreased number of red blood cells or low hemoglobin/ hematocrit level. Moreover, World Health Organization (WHO) defined severe anemia as hemoglobin level <8g/dl in all age groups other than under 5 years.[3] Prevalence of anemia was approximately high in women of reproductive age 39%, pregnant women 46% and children under 5 years 42% in 2016.[4] Prevalence of anemia is high in low income countries like Pakistan where reported prevalence of anemia was 57.7% in 2019.[5]

Anemia etiology is dependent upon whether it is hyperproliferative or hypoproliferative (corrected reticulocyte count >2% and <2% respectively). Literature reported that hypoproliferative anemia is subdivided into three main categories depending upon mean corpuscular volume (MCV); 1) microcytic anemia MCV <80fl (Iron deficiency anemia, anemia of chronic diseases, siderblastic anemia, thalassemia and lead poisoning), 2) Normocytic anemia 80-100 fl (Anemia of chronic disease, real failure, aplastic anemia, pure red cell

aplasia, myelofibrosis and multiple myeloma), 3) Macrocytic anemia MCV >100fl (Alcohol, liver disease, hypothyroidism, folate and vitamin B 12 deficiency, melody's plastic syndrome and drug induced causes).[6]

Diagnosis of anemia is usually done with peripheral blood smear or automated haematology analyzer. Peripheral blood smear is basic screening hematological tool for diagnosis, monitoring and understanding progression of anemia. The diagnostic relevance of peripheral blood smear is associated with understanding morphology of peripheral blood cells. Peripheral blood smear is still an important diagnostic tool in low and middle income countries even after advances in haematology automation and molecular techniques.[7]

Automated haematology analyzer is associated with integrated reticulocyte analysis, complete blood count analysis, reticulocyte % count, absolute reticulocyte count, reticulocyte hemoglobin content, microcytic cells %, hypochromic cells % and immature reticulocyte fraction etc.[8] Toppo et al. reported that auto analyzer is recommended as gold standard with high sensitivity 99.9% and diagnostic accuracy 80%.[9] However, limited data is available on diagnostic accuracy of peripheral blood smear. Present study aims to determine diagnostic accuracy of peripheral blood smear and automatic haematology analyzer and to determine frequency of different types of anemia diagnosed by peripheral blood smear and automatic hemolytic analyzer.

MATERIAL & METHODS
A cross sectional study was conducted at Department of Pathology, Rawal Institute of Health Sciences. Study duration was 6 months (November 2015 to April 2016). A sample size of 149 patients was calculated using 26% prevalence of anemia[10], 7% significance level and 95% confidence interval using WHO calculator. Patients were selected through non probability (consecutive) sampling. Research approval was taken from ethical review board (RHIS-REC/057/20). Inclusion criteria was based upon age >18-60 years, both genders and patients suspected for anemia. Blood

samples were drawn from each participant using aseptic precautions into ethylene diamante tetra acetic acid (EDTA) through disposable syringes. Blood samples were subjected to both testing methods (peripheral blood smear and automated haematology analysis) and hemoglobin levels in patients were measured. Patients with hemoglobin level ≤10.9g/dl were referred as anemic using Toppo et al. guidelines.[9] Data was analyzed using SPSS version 25. Mean and standard deviation was calculated for continuous data like age. Percentage and frequency was calculated for nominal and categorical data like gender and types of anemia. Chi-square test and fissure exact test was applied. We found p value ≤0.05 was considered significant.

RESULTS
Peripheral blood smear testing showed 68% sensitivity, 59% specificity, PPV 72%, NPV 55% and diagnostic accuracy 64%. Automated haematology analyzer showed 92% sensitivity, 88% specificity, 92%PPV, 88% NPV and 91% diagnostic accuracy.

ROC curve analysis showed 63% accuracy of Peripheral blood smear while 90% accuracy on automated haematology analyzer for anemia diagnosis as shown in Figure-1 and 2.

Out of 149 patients males were 42 (28.2%) and females were 107(71.8%).1. Mean age of patients was 35.1±2.1SD. There were 95 (63.8%) patients in age group 18-35 years while 54(36.2%) patients in 36-60 years age group.

Total of 112 cases showed microcytic hypochromic anemia with raised RDW (Red cell distribution width). Out of them on peripheral smear examination (17%) cases showed dimorphic blood picture. Out of 7 cases that had microcytic hypochromic anemia with Normal RDW on automated analyzer 3(42.2%) showed dimorphic blood picture on peripheral smear. Out of 11 cases having normochromic normocytic anemia with raised RDW 7(63.6%) came out to be dimorphic on peripheral smear examination. Out of 9 cases that showed normochromic normocytic anemia with normal RDW 2(22.2%)

showed dimorphic blood picture. There were 10 cases of macrocytic anemia out of which 3(30%) showed dimorphic blood picture on peripheral smear examination.

Types of Anemia	Automated Analyzer	Peripheral Smear Examination	Dimorphic Blood Picture on PF	Total	P-Value
MCHA with raised RDW	112	93	19	84	<.001
MCHA with normal RDW	07	05	03	07	<.001
NCNA with raised RDW	11	06	07	11	<.001
NCNA with normal RDW	09	07	02	09	<.001
Macrocytic anemia	10	08	03	10	<.001
Total			149		

Table-I. Comparison of results automated haematology analyzer and peripheral blood smear.

Types of Anemia	Automated Analyzer	Percentage %
MCHA with raised RDW	112	75.2
MCHA with normal RDW	07	4.7
NCNA with raised RDW	11	7.4
NCNA with normal RDW	09	6.0
Macrocytic anemia	10	6.7

Table-II. Automated analyzer findings with percentages.

Types of Anemia	MCHC on Peripheral Smear Examination	Dimorphic Blood Picture on Peripheral Smear
MCHA with raised RDW	93(83.03%)	19(17%)
MCHA with normal RDW	05(71.4%)	03(42.5%)
NCNA with raised RDW	06(54.4%)	07(63.6%)
NCNA with normal RDW	07(77.7%)	02(22.2%)
Macrocytic anemia	08(80%)	03(30%)

Table-III. Peripheral smear findings with percentages.

Diagnostic Parameters	Peripheral Blood Smear	Automated Hemolytic Analyzer
Sensitivity	68%	92%
Specificity	59%	88%
Positive predictive value	72%	92%
Negative predictive value	55%	88%
Diagnostic accuracy	64%	91%

Table-IV. Diagnostic parameters of peripheral blood smear ad automated hemolytic analyzer.
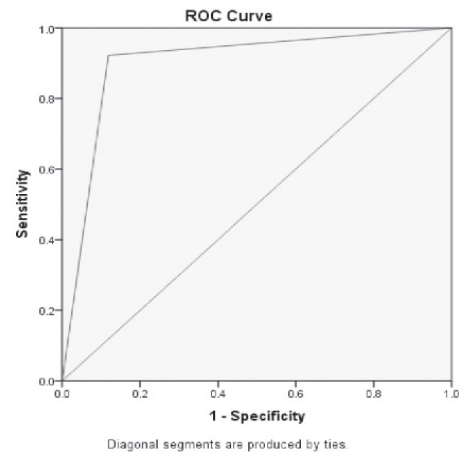

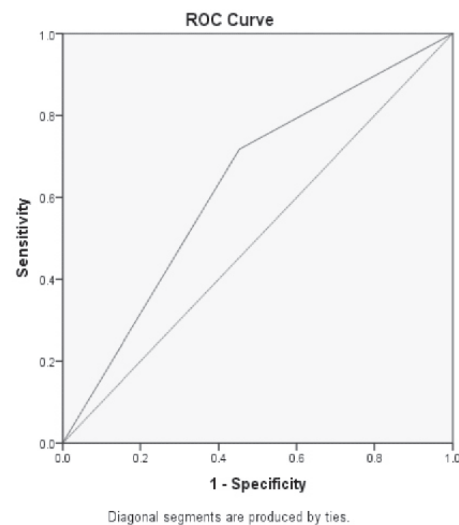**Figure-1. ROC curve analysis of peripheral Blood smear.**

Figure-2. ROC curve analysis of automated hematology analyzer.

DISCUSSION

Peripheral blood smear is an important part of

laboratory investigation for anemia diagnosis. This manual process involves tedious observation, interobserver variation and time consuming process.[11] This procedure motivated researchers to divert towards different algorithms and automated methods of diagnosis. Automated hematology analyzer is associated with accurate, rapid and avoids manual count bias.[12]

In present study, we found moderate diagnostic accuracy of peripheral blood smear 64%. Comar et al reported that accuracy of peripheral blood smear is 46.3% with 70% efficacy equivalent, 23.7% false positive and 6.73% false negative.[13] However, International society for laboratory haematology reported that efficacy of microscopic review rate is 73.8% with 10.5% false positive and 15.5% false negatives.[14] Another similar study conducted by pratumvinit et al. reported that manual peripheral smear review had 87.13% efficacy accuracy with 2.98% false negative.[15] Xing et al. reported that diagnostic efficacy of peripheral blood review rate is 50.2% with 4.2% false negative rate and 18.7% false positive rate.[16] Asad et al. reported that peripheral blood smear showed 91.3% sensitivity and 42% diagnostic accuracy with abnormal histogram.[5]

In present study, automated haematology analyzer showed 92% sensitivity and 91% diagnostic accuracy. Chapman reported that automated haematology analyzer had high sensitivity for blood cell count (CBC).[17] Another similar study reported that automated haematology analyzer is gold standard for diagnosis of blood related diseases like anemia with high diagnostic accuracy 98%.[18]

In present study, most common type of anemia diagnosed with peripheral blood smear is microcytic hypochromic anemia with raised RDW 36.7% following normchromic normocytic anemia with raised RDW 13.3% while in automated haemolytic analyzer MCHC 54.4% following NCNC with normal RDW 11.1%. Mukaya et al. reported MCHC anemia is most common anemia diagnosed by PBS and AHA 55.4% following NCNC 37.5%.[19] Kumar et al. reported that MCHC anemia 24.7% and NCNC anemia 12.64 with

PBS.[20] Sandhya I et al. reported MCHC anemia was diagnosed in 61% patients and CNCN 17% patients with PBS.[21]

Small sample size and conduction of study at single center limits generalizability of study.

CONCLUSION
Automated haemoatology analyzer had high diagnostic accuracy for diagnosis of anemia. Microcytic hypochromic anemia and normocytic normochromic are most common anemias diagnosed by peripheral blood smear and automated haematology analyzer and peripheral blood smear cannot be completely replaced by automated haemolytic analyzer. However, if both methods are used simultaneously, more accurate results can be obtained.
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