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Diagnostic Accuracy of Haematology Analyzer Sysmex Xn-1000 Scattergrams in the Diagnosis of Malaria

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Abstract

Objective: To assess the diagnostic accuracy of automated haematology analyser Sysmex XN-1000 in the diagnosis of malaria taking microscopy as gold standard.

Methodology: This Cross-sectional study was conducted at Haematology department Chughtai Institute of Pathology from 26-4-2018 to 26-10-2018. Patients were segregated for being suspicion for positivity of malaria among the cases presenting with complaint of episodes of fever. Blood sample was evaluated for presence of any microbial activity. There were two tests, which were performed on the blood sample. First the auto analyser was applied. The other sample was run through microscopic method of analysis of plasmodium and under microscope. Results were recorded and entered for further analysis. Data was stratified for age, gender, duration of symptoms. Post stratification hi square test was used taking p-value <0.05 as significant.

Results: Mean age of patients in this study was 41.21±13.77 years. Among patients 96(53.3%) were male and 84(46.7%) were females. Mean duration of symptoms was 4.01±1.99. Sensitivity and specificity of Sysmex Xn-1000 was 93.16% and 93.65% respectively and PPV and NPV was 96.46% and 88.06% respectively, overall diagnostic accuracy was 93.33%.

Conclusion: The diagnostic accuracy of automated haematology analyser sysmexXN-1000 for diagnosis of malaria was 93.33%. % so it may be concluded that the Sysmex XN-1000 analyser has an advantage in that it is able to detect unexpected malaria cases.

Key words: Diagnostic accuracy, automated haematology analyser, sysmexXN-1000, malaria, microscopy.

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Introduction

Malaria is a potentially fatal illness prevalent in tropical regions, resulting from an infection with the Plasmodium parasite, which is transmitted via the bite of an Anopheles mosquito carrying the infection. There are five plasmodium species that cause disease in human, P. Falciparum, P. vivax, P. Ovale, and P. Knowlesi. Proper identification of these malarial species is of extreme importance as falciparum can lead to fatal outcome if left untreated.[1]

Malaria is the cause of 1-3 million deaths/year. Risk factors for mortality include pregnant females, travelers who are non immune, and children age 6 months -3years. 300-500 cases of malaria are reported annually worldwide. 95% of mortality is caused by plasmodium falciparum.[2]

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Malaria has several serious complications which includes Renal failure, Hypotension and shock, Cerebral malaria, Jaundice and liver failure, Hypoglycemia, Rupture of spleen, Pulmonary edema and ARDS.

Accurate malaria diagnosis is of principal importance and is critical in its treatment and management. World Health Organization recommends that all cases be confirmed by microscopy before starting therapy. At present, many alternative methods are in use for diagnosis such as rapid diagnostic tests rapid diagnostic tests and polymerase chain reaction. Microscopy is gold standard for malaria diagnosis by detecting plasmodium species through thick (for malarial parasite identification) and thin (for species identification) peripheral smear making and detailed review of slide under microscope. Limitations of microscopy are inadequate training, poor supervision and inappropriate technology and it requires high personnel skills and experience.[4]

Rapid diagnostic tests (ICT) detect malaria antigen with antibodies that are directed against

parasite antigen on test strip. However, they are less sensitive and give false positive and false negative results and have limited shelf lives. The nucleic acid detection of malarial parasite by polymerase chain reaction is highly sensitive and specific technique but is very expensive and is not available in all laboratories.[5]

Another reliable method is the use of automated hematology analyzer Sysmex XN-1000 for diagnosing malaria. These analyzers work on the principal of flow cytometry and note scatter gram abnormalities in WBC differential area and also detect abnormal hematological parameters in malaria positive patients.[6]

Microscopic technique, apart from being gold standard has got two major pitfalls: The Peripheries are not provided well with necessary equipment to carry out the tests, and the authenticity of diagnosis relies completely on the competency of personnel performing it. The sensitivity of blood films ranges from 75–90% to as low as 50%. Modalities like Rapid diagnostic kits are often more precise than peripheral smears at predicting malarial parasite positivity, but there is wide range of variability in diagnostic sensitivity and specificity which in turn depends upon manufacturer, and are unable to ascertain with confidence as to how many parasites are present.[7]

In regions that cannot offer modern laboratory diagnostic tests, it has become common to utilize clinical history of fever as the indication to treat for malaria. A downside of this practice is over diagnosis of malaria leading to mismanagement of patient that ultimately causes wastage of resources and adds to the burden on health care system, followed by drug resistance.[8] Although PCR test have been developed, but unfortunately it is not widely available in areas where malaria is common, due to its complexity.[9]

Over a few years, with the improvement in diagnostic precision of haematology analysers, the use of fluid analysis method is proven to be effective for cytological examinationsz.[10] Cell count determination in independent body fluid examination method in haematology instruments are carried out using nucleic acid fluorescence staining techniques as well as semiconductor laser flow cytometry.[11]

Malaria can be detected in the blood smear as well as through scattergrams in hematology analyzers. Mainly two primary measurement channels in Sysmex instruments are used by which the blood parasites can be identified, given the infection is strong enough; the reticulocyte channel and the WBC differential channel. For both channels, specific fluorescence markers that tag nucleic acids are used. The more the content of intracellular nucleic acids (DNA and RNA) is, the higher the resulting fluorescence signal will be generated[12].

Methodology

In this study, the inclusion and exclusion criteria for patient selection are defined as follows:

Inclusion Criteria: Patients within the age range of 18 to 65 years, regardless of gender (including both males and females), are eligible for consideration. Suspected cases of malaria, as per the operational definition, are included. Additionally, patients who have been suspected and referred within a 7-day timeframe are eligible for participation.

Exclusion Criteria: Patients currently undergoing antimalarial therapy, as well as those experiencing diarrhea for the past 3 days upon clinical examination, are excluded from the study. Individuals with a documented history of typhoid fever within the last six months and patients currently receiving quinolone therapy for a duration of 3 days are also not considered eligible.

These criteria were established to ensure the appropriate selection of participants for the study or clinical context, facilitating clear and effective research or treatment protocols.

Patients who have been affected with HCV. After approval from the ethical committee of our lab 180 cases fulfilling the inclusion criteria were enrolled in the study. All data was collected by using a proforma. The clinical parameters like age, gender and suspected diagnosis was recorded. Patients were segregated for being suspicion for positivity of malaria among the cases presenting with complaint of episodes of fever. Blood sample was drawn in a 3cc syringe and was evaluated for the presence of any microbial activity. There was two test, which was performed on the blood sample. First the auto analyser was applied for the detection of malarial parasite through abnormal scattergrams in DIFF, WBC/BASO channel. Once the microbial presence or absence is noted, the other sample was run through microscopic method of analysis of plasmodium and its species by thick and thin

film making and evaluation of slide under microscope. Results obtained from both the methods were recorded and entered for further analysis. All the information was recorded on a pre designed proforma. (Annexed-I)

Data was analysed using SPSS version 23. The mean and standard deviation was calculated for quantitative variables including age and duration of symptoms. The qualitative variables including gender, diagnosis of malaria on the both tests were presented in frequencies and percentages. 2X2 table was generated to calculate sensitivity, specificity, positive predictive value and negative predictive value of sysmex-1000 keeping microscopy as gold standard. Data was stratified for age, gender, duration of symptoms. Post stratification hi square test was used taking p-value ≤ 0.05 as significant.

Results

Mean age of patients in this study was 41.21±13.77 years. Among patients 96(53.3%) were male and 84(46.7%) were females. Mean duration of symptoms was 4.01±1.99. Minimum and maximum duration of symptoms was 1 and 7 days respectively.

Results of Sysmex Xn-1000 showed 113(62.8%) positive patients and 67(37.2%) negative patients. However, microscopy findings showed 117(65%) positive and 63(35%) negative patients.

Sensitivity and specificity of Sysmex Xn-1000 was 93.16% and 93.65% respectively and PPV and NPV was 96.46% and 88.06% respectively, overall diagnostic accuracy was 93.33%. Table I

Table I: Diagnostic Accuracy of Sysmex Xn-1000 for Malaria Diagnosis taking microscopy as Gold Standard.

		Microscopy		Total
		Yes	No	
Sysmex Xn-100	Yes	109(93.2%)	4(6.3%)	113
	No	8(6.8%)	59(93.7%)	67
Total		117	63	180

Sensitivity= 93.16%
Specificity= 93.65%
Positive Predictive Value= 96.46%
Negative Predictive Value= 88.06%
Diagnostic Accuracy= 93.33%

Highest sensitivity and specificity was seen in elderly patients who were >55 years and in age group 36-55 years. Patients in the age group 36-55 years had the highest value for PPV and highest NPV was seen in patients in age group >55 years. Table-II

Table II: Diagnostic Accuracy of Sysmex Xn-1000 for Malaria Diagnosis taking microscopy as Gold Standard stratified for age.

	18-35	36-55	>55
Sensitivity	92.50%	90.74%	100%
Specificity	92.31%	95.65%	92.86%
Positive Predictive Value	94.87%	98%	95.83%
Negative Predictive Value	88.89%	81.48%	100%
Diagnostic Accuracy	92.42%	92.21%	97.30%

All diagnostic accuracy parameters i.e. Sensitivity, Specificity, PPV and NPV were higher for male patients. Table III

Table III: Diagnostic Accuracy of Sysmex Xn-1000 for Malaria Diagnosis taking microscopy as Gold Standard stratified for gender.

			Microscopy		Total
			Yes	No	
Sysmex Xn-100	Male	Yes	58 (95.1%)	1 (2.9%)	59
		No	3 (4.9%)	34 (97.1%)	37
	Female	Yes	51 (91.1%)	3 (10.7%)	54
		No	5 (8.9%)	25 (89.3%)	30

Diagnostic accuracy parameters	Male	Female
Sensitivity	95.08%	91.07%
Specificity	97.14%	89.29%
Positive Predictive Value	98.31%	94.44%
Negative Predictive Value	91.89%	83.33%
Diagnostic Accuracy	95.83%	90.48%

Patients with short duration of symptoms among them sensitivity, Specificity, PPV and NPV was 95.35%, 90.62%, 93.18%, 93.55% and 93.33% respectively. However, patients who had a bit longer duration of symptoms among them sensitivity, Specificity, PPV and NPV was 91.89%, 96.77%. 98.55% and 83.33% respectively. Table IV

Discussion

The Sysmex analyzer works on flow cytometry principles, utilizing a semiconductor laser to deliver

Table IV: Diagnostic Accuracy of Sysmex Xn-1000 for Malaria Diagnosis taking microscopy as Gold Standard stratified for duration of symptoms

			Microscopy		Total
			Yes	No	
Sysmex Xn-100	1-3 Days	Yes	41(95.3%)	3(9.4%)	44
		No	2(%4.7)	29(90.6%)	31
	4-7 Days	Yes	68(91.9%)	1(3.2%)	69
		No	6(8.1%)	30(96.8%)	36
Diagnostic accuracy parameters					
			1-3 Days	4-7 Days	
Sensitivity			95.35%	91.89%	
Specificity			90.62%	96.77%	
Positive Predictive Value			93.18%	98.55%	
Negative Predictive Value			93.55%	83.33%	

three forms of optical data about cells. It measures cell size using forward scatter light, the complexity of internal structure such as granules using side scatter light, and nuclear content using side fluorescence light.[13]

When malarial parasites detoxify the free heme that is generated after the breakdown of hemoglobin, hemozoin, a crystalline brown pigment, is created. By phagocytosing hemozoin, neutrophils and monocytes make it possible to automatically detect malaria infections using abnormal scattergrams. [14]

The current study revealed abnormalities in hematological parameters such as anemia, leukopenia, and thrombocytopenia, consistent with prior research. In a study by Sharma et al., the most prevalent scattergram anomalies observed in P. vivax samples were greying of eosinophil and neutrophil groups (47.05%), rightward shift of RBC ghost area (32.4%), and the presence of two neutrophil populations (15.7%).[14]

Mohapatra et al.'s study reported a sensitivity of 74.2% and specificity of 91.1%, while Sharma et al.'s study showed a sensitivity of 83.78% and specificity of 94.82%. 15 Other studies yielded similar findings, with sensitivity at 80% and specificity at 93.6%. In comparison to immune chromatographic studies, these results indicate lower specificity and sensitivity.[16]

Whereas in our study there was sensitivity and specificity of 93.16% and 93.65% which is almost similar to the study of Sharma et al and different from the findings of Mohapatra et al as discussed above.

In a retrospective analysis of complete blood counts (CBCs) conducted using Sysmex Xn-1000 and XE-5000 hematology analyzers, 67 cases of malaria imported from malaria-endemic regions to metropolitan France (a non-endemic country) were examined. The findings revealed that, concerning malaria diagnoses, the standard white blood cell (WBC) scattergrams displayed distinctive abnormalities, resembling those observed in patients diagnosed in endemic countries. These abnormalities exhibited a sensitivity of 83% and a specificity exceeding 99%, ensuring precise and reliable diagnostic accuracy.

Few more studies reported sensitivity ranged from 81.8 to 98% and specificity from 72.3% to 96.9%. While in our study the reported sensitivity and specificity was 93.16% and 93.65% respectively which is in range of above mentioned studies. Automated blood cell analysers can detect cases of malaria in the absence of clinical suspicion. There are several evidences regarding the use of these analysers for detection of malaria. If the consensus guidelines for slide review of automated analyzer results, as outlined by the International Society for Laboratory Hematology, had been followed, there would have been no need for microscopic review. However, in stark contrast, other studies examining 49 patients with malaria infection failed to identify any distinctive changes in routine complete blood count (CBC) analysis or routine differential scattergrams that could suggest malaria (with a sensitivity of 0%).

A recently published diagnostic model designed for detecting malaria infection, which incorporates both routine and research parameters from the Sysmex XN-1000 Hematology Analyzer (HA), has achieved an impressive accuracy rate of 77.1% in identifying cases.[17] The Sysmex XN-1000 HA has been

useful for diagnosing malaria in areas where the disease is endemic, according to numerous writers. What is notable is that this analyzer can offer indicators that are distinct enough to demand attention and are predictive of a malaria diagnosis.

Numerous pieces of evidence support the use of these analyzers for malaria detection. While an abnormal White Blood Cell (WBC) scattergram generated by the Sysmex XN-1000 analyzer is highly specific for diagnosing malaria, it remains impractical to scrutinize every WBC histogram manually in a laboratory setting for malaria detection. Additionally, a high incidence of thrombocytopenia has been observed in malaria patients.

The need for specialized knowledge and continual training is one of the drawbacks of using a microscope to examine stained slides in order to find malaria parasites. In order to raise suspicion of a malaria infection based on a high eosinophil count or uncounted eosinophils before the slide is inspected, the Sysmex XN-1000 has an advantage over microscopic examination.[18]

Conclusion

The diagnostic accuracy of automated haematology analyser sysmexXN-1000 in the diagnosis of malaria taking microscopy as gold standard was 93.33% so it may be concluded that the Sysmex XN-1000 analyser has an advantage in that it is able to detect unexpected malaria cases. Considering its high sensitivity, haematological analysis performed with this instrument can be considered an alternative to existing methods for the diagnosis of malaria.

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