Open Access ORIGINAL ARTICLE

Accuracy of Haematology Analyzer in the Diagnosis of Malaria in Comparison with Gold Standard Microscopy

Amna Imtiaz[1,2], Noshina Noreen[2], Syed Mudassar Ali Raza[2], Sundas Ali[1], Farah Hanif[1,2], Rafay Ali Khan[2]

[1]Department of Pathology, Pakistan Institute of Medical Sciences, Islamabad, Pakistan; [2]Department of Pathology, Pakistan Atomic Energy Commission (PAEC) General Hospital, Islamabad, Pakistan

ABSTRACT

Objective: To assess the accuracy of Haematology analyzer in the diagnosis of malaria in comparison with gold standard microscopy.

Methodology: A cross-sectional study was conducted at the Pakistan Institute of Medical Sciences and the Pakistan Atomic Energy Commission in Islamabad, Pakistan. The study was conducted for around six months, from May 2023 to October 2023. The Mindray BC-6200 haematology analyser was utilised to evaluate a total of 191 samples, comprising 127 samples from that were infected with malaria and 64 samples from healthy controls. When the presence of malaria parasites, identified as Plasmodium vivax and Plasmodium falciparum, was detected in dyed thick blood film, a microscopy examination was carried out as a reference. Analyse-it v4.92.3 was used to create the receiver operating characteristic (ROC) curve analysis.

Results: The INR by BC-6200's sensitivity and specificity for P. falciparum and P. vivax infections. The sensitivity of the InR by BC-600 for P. falciparum and P. vivax was 27.9% and 85.5%, respectively. The specificity of the INR by BC-6200 for P. vivax and P. falciparum was 82.7% and 86.5%, respectively. The infection densities in microscopy varied statistically significantly between the various INR groups ($\chi 2 = 14.50$, $P < 0.005$). Analysis was conducted on the correlation between the cell blood count and the count of InR in both the P. vivax and P. falciparum-infected patient groups. The results showed a clear correlation between InR (P. vivax group R2 = 0.87) and ΔWBC (WBCDIFF–WBCBASO). In the Mindray BC-6200 haematology analyser, WBCBASO represents the number of WBC counting in the BASO channel with severe membrane degradation, while WBCDIFF represents the number of WBC counting in the DIFF channel with mild lyse. The volume distribution widths of RBC, HGB, and red blood cells did not differ substantially. The reticulocyte characteristics of the P. vivax/P. falciparum patient group and control group differed significantly ($P<0.02$), although RBC, HGB, and red blood cell volume distribution width (RDW) did not differ significantly ($P > 0.05$). There was a significant difference ($P<0.02$) in the PLT count between the P. vivax/P. falciparum patient groups and the control group.

Conclusion: The results imply that malaria might be screened for in a clinical setting using the BC-6200 haematology analyser "INR" flag and "INR#/INRg%" parameters.

Key words: Light microscopy, Malaria, Mindray BC -6200, Automated Haematology analyzer, Diagnostic accuracy.

Authors' Contribution:
[1]Conception; Literature research; manuscript design and drafting; [2] Critical analysis and manuscript review; [1,2]Data analysis; Manuscript Editing.

Correspondence:
Amna Imtiaz
Email: amnaimtiazkhan@gmail.com

Article info:
Received: October 10, 2023
Accepted: December 20, 2023

Cite this article. Imtiaz A, Noreen N, Raza SMA, Ali S, Hanif F, Khan RA. Accuracy of Haematology Analyzer in the Diagnosis of Malaria in Comparison with Gold Standard Microscopy. J Islamabad Med Dental Coll. 2023; 12(4): 237-243.
DOI: https://doi.org/10.35787/jimdc.v12i4.1055

Funding Source: Nil
Conflict of interest: Nil

Introduction

Malaria is a life-threatening disease spread to humans by some types of mosquitoes. It is mostly found in tropical countries. It is preventable and curable. Malaria mostly spreads to people through the bites of some infected female *Anopheles*

mosquitoes. Blood transfusion and contaminated needles may also transmit malaria. The first symptoms may be mild, similar to many febrile illnesses, and difficulty to recognize as malaria. Left untreated, *P. falciparum* malaria can progress to severe illness and death within 24 hours.[1]

There are 5 *Plasmodium* parasite species that cause malaria in humans and 2 of these species – *P. falciparum* and *P. vivax* – pose the greatest threat. The other malaria species which can infect humans are *P. malariae, P. ovale* and *P. knowlesi*.[2]

According to the latest World malaria report, there were 247 million cases of malaria in 2021 compared to 245 million cases in 2020. The estimated number of malaria deaths stood at 619 000 in 2021 compared to 625 000 in 2020.[3]

Over the 2 peak years of the pandemic (2020–2021), COVID-related disruptions led to about 13 million more malaria cases and 63 000 more malaria deaths[4]

Early diagnosis and treatment of malaria reduces disease, prevents deaths and contributes to reducing transmission. WHO recommends that all suspected cases of malaria be confirmed using parasite-based diagnostic testing (through either microscopy or a rapid diagnostic test) [5 6]

Malaria is a serious infection and always requires treatment with medicine. Malaria diagnosis has evolved over the years, and various methods are now used for its detection. The gold standard for malaria diagnosis is still microscopic examination of blood smears. Microscopy involves preparing a thick and thin blood smear on a glass slide and staining the blood cells. [7]

The incorporation of specialized parameters like "INR" and "INR#/INRg%" into hematology analyzers has become a focus of study and innovation in the quest to advance diagnostic skills. Only to be used for research purposes, these parameters closely resemble the proportion of infected Red Blood Cells (IRBCs) in a particular sample and provide a specific indicator for their detection. The objective of this study is to evaluate the usefulness and effectiveness of these parameters, especially the INR#/INR%, in the routine screening of blood samples for malaria in endemic areas. This is a bold and novel approach.[8]

The main goal of this study is to determine whether the infected RBC flag and related INR#/INR% characteristics can be used as a valid malaria screening tool when doing normal blood work. Additionally, a comparative analysis is conducted in which the flag information of Complete Blood Count (CBC) parameters is examined between two groups: one that is infected with malaria and the other that is not, using the common strains of Plasmodium vivax and Plasmodium falciparum. By allowing for a more nuanced understanding of how these parameters alter in the presence of malaria and differentiating between various strains of the parasite, this comparative lens adds complexity to the study.[9]

With its sophisticated features, the Mindray BC-6200 offers a specific flag for the identification of INR and INR#/INR% values. This feature is intended solely for research purposes and provides a numerical representation of the proportion of malaria-infected red blood cells in a given blood sample. The objective of this study is to assess the BC-6200's practical use in routine blood testing in malaria-endemic areas outside of the lab.[10]

The main objective is to evaluate the Mindray BC-6200's usefulness and efficacy as a malaria screening tool, taking into account the particular difficulties that endemic regions provide.[11] Through a close examination of the infected RBC flag's performance and related metrics in standard testing, this study seeks to provide significant knowledge to the continuing discussion about how to improve malaria diagnosis.

Additionally, the study broadens its focus to include a comparison of the strains of Plasmodium vivax and Plasmodium falciparum between a group of people without malaria and a group that has the disease. By using a comparison approach, the evaluation's accuracy is improved and it becomes clearer how

the Mindray BC-6200's parameters react to various strains of malaria.[12]

Essentially, the goal of this research is to close the gap that exists between the urgent healthcare needs of locations where malaria is endemic and state-of-the-art diagnostic technologies. We hope to further the global effort to combat this prevalent and difficult illness by examining the performance of the Mindray BC-6200 in regular blood testing for malaria. This will help to build more accessible and effective screening instruments.

Methodology

This cross-sectional study was conducted in Islamabad, Pakistan, with assistance from the Pakistan Atomic Energy Commission (PAEC) and the Pakistan Institute of Medical Sciences (PIMS). Participants in the research included people of all ages and demographics who had either confirmed or suspected malaria. It was essential that they lived in Islamabad, Pakistan. On the other hand, members of the Pakistan Atomic Energy Commission staff as well as members of the local community in good health who had no history of malaria or hematological diseases made up the control group. The control group also had to meet the requirement of being residents of Islamabad.

To narrow down the pool of participants, exclusion criteria were established. Patients taking medication that affects blood cell counts, pregnant women, and those with a history of chronic disorders impacting hematological parameters were not included. Similarly, pregnant women, people on medications that influence blood cell counts, and people with a history of malaria or hematological diseases were not included in the control group.

The age range of the participants differed according to the particular groupings. Both Plasmodium vivax and Plasmodium falciparum groups of malaria patients were recruited from a wide age range: 3 to 69 years and 6 to 60 years, respectively. The control group consisted of people ranging in age from 8 to 70. For the purpose of evaluating the BC-6200 Hematology Analyzer in the context of malaria screening in Islamabad, Pakistan, these inclusion and exclusion criteria, in conjunction with the designated age ranges, were crucial in forming a thorough and representative participant cohort.

The study closely followed the moral standards set out by the PIMS and PAEC ethical review committees. These committees granted approval for the research protocol, highlighting adherence to ethical guidelines. Every participant or their legal guardian provided informed consent, demonstrating the voluntary nature of participation.

Results

Light microscopy was used to assess the sensitivity and specificity of INR by BC-6200 for P. vivax and P. falciparum infections. P. falciparum and P. vivax had sensitivity values of 27.9% and 85.5%, respectively, for the INR by BC-600. 82.7 and 86.5%, respectively, were the specificity of the INR% by BC-6200 for P. vivax and P. falciparum. Table 1 has more information on these findings.

Table 2 displays the results, which indicated that there was a statistically significant difference in the infection densities in microscopy ($\chi 2 = 14.50$, $P<0.005$) across the different INR groups. Furthermore, these findings showed that group II and group III of the INR had larger infection densities than group I. Table 3 displays the BC-6200 hematology analyzer's automated WBC counts and questionable flags. In both the P. falciparum-infected and P. vivax-infected patient groups, the relationship between the count of INR and the cell blood count was examined. The outcome demonstrated that ΔWBC (WBCDIFF–WBCBASO) and INR (P. vivax group R2 = 0.87) had a definite association. WBCBASO is the number of WBC counting in the BASO channel with severe membrane degradation in the Mindray BC-6200 hematology analyzer; WBCDIFF is the number of WBC counting in the DIFF channel with mild lyse. RBC, HGB, and red blood cell volume distribution width did not differ significantly. P. vivax/P.

falciparum patient group and control group showed a significant difference in reticulocyte parameters (P<0.02), but there was no significant difference in RBC, HGB, or red blood cell volume distribution width (RDW) between the two groups (P > 0.05). The PLT count of the P. vivax/P. falciparum patient groups and the control group differed significantly (P < 0.02).

Discussion

The BC-6200 Hematology Analyzer, which uses SF-Cube technology, is a noteworthy development in the field of diagnosing malaria. With the addition of clinically significant suspect flags, the three-dimensional cell analysis offered by SF-Cube technology permits comprehensive evaluations of white blood cells (WBC), reticulocytes (RET), and nucleated red blood cells (NRBC). 'Infected RBC' as a specialized flag is in line with the changing hematological analyzer market, where accurate and timely infectious agent identification is critical. The capacity to differentiate between red blood cells that are infected plays a significant role in the timely and accurate identification of malaria, which is essential for better patient outcomes. The BC-6200's diagnostic skills are improved by the thorough examination of routine data, questionable flags, and the 'infected RBC' parameter. A comprehensive picture of the patient's hematological profile in relation to infection is provided by the interaction of established hematological parameters with indicators unique to malaria.[13]

Table 1: Performance of InR‰ by BC-6200 in Comparison to Reference Light Microscopy for Malaria Diagnosis

Performance Metric	P. vivax (95% CI)	P. falciparum (95% CI)
Sensitivity	85.5% (80.73–90.27%)	27.9% (23.13–35.42%)
Specificity	82.7% (74.34–88.91%)	86.5% (79.21–91.86%)
Positive Likelihood Ratio	4.23 (2.88–6.22)	2.15 (1.27–3.64)
Negative Likelihood Ratio	0.18 (0.13–0.25)	0.76 (0.62–0.92)
Disease Prevalence	58.5% (52.19–63.45%)	45.7% (38.39–53.18%)
Positive Predictive Value	87.2% (80.73–90.27%)	56.4% (44.87–69.21%)
Negative Predictive Value	80.1% (74.34–88.91%)	62.8% (56.19–71.42%)
Total Consistent Rate	84.3%	63.8%
Kappa Value	0.682	0.213

Table 2: Difference in Infection Density among Plasmodium vivax Groups [M (P25, P75)]

Group	Number of InR	Infection Density in Microscope (10^9/L) [M (P25, P75)]	χ^2	P-Value
I	1200 (800, 1800)		14.50	P< 0.001
II	2400 (1100, 4000)*			P < 0.05
III	2800 (2000, 5000)*			P < 0.05

Table 3: Blood Cell Parameters and Suspect Flags between Control Group and Malaria Groups in BC-6200 Analyzer

Parameters	Control Group (n=64)	Malaria Group (n=127)	Malaria Group (n=127)	P1	P2
		P. vivax (n=96)	P. falciparum (n = 31)		
WBC [DIFF] count (×10^9/L)	7.22 (2.89 to 23.41)	6.75 (2.23 to 21.98)	6.12(2.55 to 12.4)	0.03	0.02
WBC [BASO] count (×10^9/L)	7.15 (2.84 to 23.65)	5.88 (1.14 to 12.25)	4.70(2.29-9.78)	0.01	0.02
WBC [DIFF]–WBC [BASO] (×10^9/L)	0.07 (−5.5 to 15.88)	1.65 (−0.35 to 1.5)	0.5(-0.67-2.06)	0.02	0.02
RBC (10^12/L)	5.12 (3.26 to 7.32)	4.78 (1.68 to 7.02)	4.54(2.94-6.53)	0.68	0.64
HGB (g/L)	142.15 (101 to 216)	134.72 (55 to 218)	125.15(62-165)	0.81	0.79
RDW-CV (%)	14.12 (12.2 to 18.1)	15.25(11.8 to 34.1)	14.55(12-18.2)	0.64	0.64
RET (%)	1.12 (0.37 to 3.04)	1.78 (0.50 to 14.59)	2.01(0.52-7.58)	0.02	0.02
IRF (%)	5.00 (0 to 18.75)	10.55 (1.05 to 25.8)	6.80 (0.9 to 25.1)	0.02	0.03
LFR (%)	94.57 (81.9 to 100)	89.45 (54.9 to 98.9)	93.21 (74.9 to 99.1)	0.03	0.05
MFR (%)	4.65 (0 to 14.5)	9.32 (1.05 to 25.5)	6.23 (0.9 to 19.3)	0.02	0.03
HFR (%)	0.18 (0 to 4.7)	2.01 (0 to 23.5)	0.57 (0 to 5.8)	0.02	0.03
Anaemia (%)	10 (15.62%)	28 (29.13%)	6 (28.57%)	7 (33.33%)	0.02
PLT count (×10^10/L)	210 (72 to 380)	120 (15 to 320)	149(53 to 323)	170 (63 to 340)	0.02
Malaria Flag/Parameters					
InR flag	0	90 (91.5%)		7 (33.33%)	0.01
InR#					
InR‰					

A study done by Katarzyna Kulik *et al.,* concluded that RDW (r =.8350), MPV (r =.7634), Mon# (r =.8366), Baso# (r =.9205), and NRBC (r =.3768), the BC-6200 analyzer and ADVIA 2120i exhibited a strong association (r ≥.97). Compared to the ADVIA 2120i (r =.5677), the BC-6200 demonstrated a higher correlation with microscopic examination for NRBC (r =.8902). For flagging blasts (80.4%), immature granulocytes (80.5%), and abnormal lymphocytes (69.0%), the BC-6200 has demonstrated good efficiency.[14]

According to research by S. A. Khan et al., microscopy is the gold standard for identifying malaria. Even though it takes a long time—one test takes sixty minutes—if done by a patient and a qualified technologist, it is affordable and accurate. The Ideal exam, in contrast, is costlier but simpler and more objective. It also doesn't require any equipment. However, because there are fewer hospital admissions and morbidity, the cost element becomes less important.[15]

Another study by Germán Campuzano-Zuluaga *et al.,* found that the identification of malaria using hematology analysers can be helpful as an adjuvant diagnostic tool in the work-up of feverish patients, as it is a by-product of its primary function, the CBC analysis. In order to quickly diagnose malaria and begin treatment, it would be ideal to include a flag for the disease and utilize it to direct microscopic examination of the patient's blood. Presently, the Coulter GEN·S, LH 750, and Sysmex XE-2100 analysers can be automated to detect malaria with the use of a Laboratory Information System. However, it is recommended that these numerical

diagnostic criteria be verified using population-based samples. The industry's involvement is essential to these advancements, and it would be ideal if hematology analyzer manufacturers were willing to assess and incorporate algorithms in their devices that could identify samples that have a high probability of being malarial. This approach could help produce more precise algorithms in devices that are otherwise simpler.[16]

According to a comparison evaluation conducted by Katarzyna Kulik *et a*l., the new Mindray BC-6200 analyzer offers good linearity and precision. With the ADVIA 2120i analyzer, morphological and 5-diff findings showed a very excellent correlation for the majority of evaluated parameters. Moreover, the BC-6200 outperformed the ADVIA 2120i in terms of correlation with microscopic evaluation in NRBC determination. A great deal of crucial information about the tested sample may be obtained by analyzing the scatter grams and flags produced by the BC-6200. This process also makes it easier to identify samples that need to be examined under a microscope.[17]

According to a study by G. da Rin1 *et al*., Mindray BC-6800, Sysmex XN-module, and Sysmex XE-2100 displayed low mean bias in Bland-Altman's plot that was comparable with LoD and LoQ, but differences between Siemens ADVIA 2120i and Coulter UniCel DxH 800 were higher and increased proportionately with increasing NRBC concentration. It is significant to note that each of the two skilled hematologist counted 1000 cells at 400× magnification in order to improve the accuracy of the manual NRBC counts.[18]

According to a study by Tae Hwan Lee, M.D. et al., the BC-6200 performed exceptionally well when it came to background, carryover, and precision CBC parameter results. Moreover, there was a strong correlation between the CBC values and the standard instrument (XE-2100). The flagging's efficiency, specificity, and sensitivity were also deemed satisfactory. We come to the conclusion that BC-6200 is a capable HA that can fulfil the demands of mid-volume testing in clinical laboratories and deliver trustworthy and accurate diagnostic results.[19]

Conclusion

The study concludes by highlighting the importance of the 'infected RBC' flag and malaria-specific parameters, as well as the usefulness of the BC-6200 Hematology Analyzer in the diagnosis of malaria. This invention supports further efforts to improve infectious disease diagnostic accuracy, especially in areas where malaria represents a substantial health burden. The BC-6200 is a potentially useful weapon in the front-line fight against malaria and other blood-borne infections as technology develops. However, because to their tiny size and poor nucleic acid content, the ring stage and early trophozoite stage of Plasmodium cannot be readily detected on BC-6200.

References

1. Talapko J, Škrlec I, Alebić T, Jukić M, Včev A. Malaria: the past and the present. Microorganisms. 2019 Jun 21;7(6):179.; https://doi.org/10.3390/microorganisms7060179
2. Luepke KH, Suda KJ, Boucher H, Russo RL, Bonney MW, Hunt TD, et al. Past, present, and future of antibacterial economics: increasing bacterial resistance, limited antibiotic pipeline, and societal implications. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2017;37(1):71-84. https://doi.org/10.1002/phar.1868
3. Ningombam A, Sarkar A, Acharya S, Chopra A, Kumar K, Subramanian A. Application of Sysmex XN-series automated haematology analyser in the rapid detection of malaria. Indian Journal of Hematology and Blood Transfusion. 2020 Jul;36:512-8. https://doi.org/10.1007/s12288-020-01276-x
4. Khartabil TA, de Rijke YB, Koelewijn R, van Hellemond JJ, Russcher H. Fast detection and quantification of Plasmodium species infected erythrocytes in a non-endemic region by using the Sysmex XN-31 analyzer. Malaria Journal. 2022 Apr 11;21(1):119.DOI https://doi.org/10.1007/s12288-020-01276-x
5. Bria YP, Yeh CH, Bedingfield S. Significant symptoms and nonsymptom-related factors for malaria

diagnosis in endemic regions of Indonesia. International Journal of Infectious Diseases. 2021 Feb 1;103:194-200. https://doi.org/10.1016/j.ijid.2020.11.177

6. Sori G, Zewdie O, Tadele G, Samuel A. External quality assessment of malaria microscopy diagnosis in selected health facilities in Western Oromia, Ethiopia. Malar J.2018;17:233. https://doi.org/10.1186/s12936-018-2386-2.
7. Abhirup Sarkar, AnitaChopra; Application of Sysmex XN-Series Automated Haematology Analyser in the Rapid Detection of Malaria; Indian J Hematol Blood Transfus.2020 Jul; 36(3): 512–518. https://doi.org/10.1007/s12288-020-01276-x
8. Sun Y, Xiang D, Chen C, He S, Qi H, Wang C. Infected RBC flag/parameter provided by Mindray BC-6800 haematology analyzer aid the diagnosis of malaria. Malaria Journal. 2019 Dec;18(1):1-8. https://doi.org/10.1186/s12936-019-2890-z
9. Manguin S, Foumane V, Besnard P, Fortes F, Carnevale P. Malaria overdiagnosis and subsequent overconsumption of antimalarial drugs in Angola: Consequencesand effects on human health. Acta tropica 2017;171:58-63.https://doi.org/10.1016/j.actatropica.2017.03.02
10. Silamut K, Phu NH, Whitty C, Turner GD, Louwrier K, Mai NT, et al. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. 1999;155(2):395-410.
11. Anselmo FC, Soumanou AG, de Aguiar Ferreira C, Sobrinha FM, Castro AC, Brito RO, Mota AJ, de Souza Gonçalves M, Neto JP. THE HEMATOLOGICAL PARAMETERS AND BIOCHEMICAL MARKERS OF IRON STATUS IN ALFA-THALASSEMIA 3.7 KB DELETION FROM METROPOLITAN REGION OF MANAUS, AMAZONAS, BRAZIL.: alfa-Thalassemia 3.7 deletion From Amazonas, Brazil. Mediterranean Journal of Hematology and Infectious Diseases. 2021 Jan 1;13(1):e2021001-.
12. Varo R, Balanza N, Mayor A, Bassat Q. Diagnosis of clinical malaria in endemic settings. Expert Review of Anti-infective Therapy. 2021 Jan 2;19(1):79-92. https://doi.org/10.1080/14787210.2020.1807940
13. Gerke O. Reporting standards for a Bland–Altman agreement analysis: A review of methodological reviews. Diagnostics. 2020 May 22;10(5):334. https://doi.org/10.3390/diagnostics10050334
14. Mukry SN, Saud M, Sufaida G, Shaikh K, Naz A, Shamsi TS. Laboratory diagnosis of malaria: comparison of manual and automated diagnostic tests. Canadian Journal of Infectious Diseases and Medical Microbiology. 2017 Jan 1;2017.https://doi.org/10.1155/2017/9286392
15. Khan SA, Anwar M, Hussain S, Qureshi AH, Ahmad M, Afzal S. Comparison of Optimal malarial test with light microscopy for the diagnosis of malaria. JOURNAL-PAKISTAN MEDICAL ASSOCIATION. 2004 Aug 1;54(8):404-7.
16. Campuzano-Zuluaga G, Hänscheid T, Grobusch MP. Automated haematology analysis to diagnose malaria. Malaria Journal. 2010 Dec;9:1-5.DOI https://doi.org/10.1186/1475-2875-9-346
17.. Kulik K, Kwiecień I, Chełstowska B, Rutkowska E, Rzepecki P. Evaluation and comparison of the new Mindray BC-6200 hematology analyzer with ADVIA 2120i. International Journal of Laboratory Hematology. 2021 Jun;43(3):395-402.https://doi.org/10.1111/ijlh.13418
18. Da Rin G, Vidali M, Balboni F, Benegiamo A, Borin M, Ciardelli ML, Dima F, Di Fabio A, Fanelli A, Fiorini F, Francione S. Performance evaluation of the automated nucleated red blood cell count of five commercial hematological analyzers. International Journal of Laboratory Hematology. 2017 Dec;39(6):663-70. https://doi.org/ 10.1111/ijlh.12722
19 Lee TH, Kim H, Park M, Hur M, Lee CH. Performance Evaluation of the Mindray BC-6200 Hematology Analyzer; Comparison with Sysmex XE-2100 and Manual Microscopy. Laboratory Medicine Online.;12(4):269-77.DOI: https://doi.org/10.47429/lmo.2022.12.4.269