Original Article

Host Haematological Indices: Potential Diagnostic Markers in Plasmodium Vivax Malaria

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Objective: 1. To identify the fluorescent signal pattens on WBC histograms in flowcytometry based, five parts differential haematology analyzers, in smear positive cases. 2. To make a diagnostic algorithm based on haematological parameters and WBC scattergrams in suspicious cases of P vivax malaria.

Methods: Seventy smear positive Plasmodium vivax haematological indices on automated 5 part differential hematology analyzers (XE2100 & XE5000) were checked for seminal use as potential diagnostic markers.

Results: Seventy smear positive Plasmodium vivax malaria cases were selected. Haematological indices revealed that 83.4% had thrombocytopenia. Pseudoeosinophilia was seen in 76.4% cases with 51/68 showing more than 5% gap. Considerable anaemia (Hb <10 g/dl) was exhibited by 32.8% of the patients. Leukopenia was seen only in 11 cases. Monocytosis was seen in 30 patients

Conclusion: CBC run on automated analyzers gives information in the form of an integrated pattern of results. When thrombocytopenia along with raised MPV & PDW, anemia, leukopenia, monocytosis, eosinophilia (Pseudoeosinophilia in actual), abnormalities of WBC scatter-grams, monocytosis and altered RDW are read in collaboration lead to strong suspicion of P vivax malaria infestation.

Keywords: Plasmodium vivax malaria, automated hematology analyzers, hematology scatter grams, thrombocytopenia, pseudoeosinophilia, monocytosis

Introduction

Malaria is caused by the bite of female Anopheles mosquito, transmitting a protozoan, namely, Plasmodium. Four species of plasmodium cause malaria in humans: Plasmodium vivax (P vivax), Plasmodium falciparum, Plasmodium ovale, and Plasmodium malariae.[1] In 2013, there were an estimated 584000 malaria deaths worldwide (95% uncertainty interval, 367 000755 000). Pakistan falls in range of 10-49 deaths per 100,000 of population. About 80% of estimated malaria cases in 2013 occurred in just 18 countries and 80% of deaths in 16 countries. For P vivax cases, three countries (India, Indonesia, and Pakistan) accounted for more than 80% of estimated cases. The global burden of mortality and morbidity was dominated by countries in sub-Saharan Africa.[2] Usual presentation of the individuals with malaria is fever, chills, sweating, headache, vomiting, diarrhea, abdominal pain and distension, cough splenomegaly and hepatomegaly.[3] General work up of malaria includes blood counts, peripheral smear for malaria, urine examination, liver and renal function tests, CSF analysis, and immunochromatography for malarial antibodies depending upon the clinical history of the patient. Other more specialized techniques involve ELISA for malarial antibodies, detection of malarial DNA by PCR, histological detection on biopsies, and detection of malarial LDH by Gel Agglutination technique.[4]

Globally malaria is responsible for a lot of mortality (5,84000 in 2013) and morbidity(198 million cases in 2013).[2] It is most prevalent in rural tropical areas below elevations of 1000 m (3282 ft). P vivax is distributed widely but it causes less morbidity and mortality. Anemia in malaria is usually caused by hemolysis due to direct invasion of red cells, anemia of chronic disease, hypersplenism, hemophagocytic syndrome and erythrophagocytosis, dyserythropoiesis, immune hemolysis and cytokine dysregulation. Thrombocytopenia is mainly due to direct infection of platelets and increased sequestration in the presence of palpable splenomegaly and circulating immune complexes. Disseminated intravascular coagulation may also contribute in some cases towards severe thrombocytopenia.[5] Hematological manifestations of plasmodium Vivax infection include thrombocytopenia, anemia, leukopenia, leukocytosis, alterations in platelet indices (Mean Platelet Volume-

MPV, and Platelet distribution width-PDW), moncytosis, pseudoeosinophilia, abnormal scatter-grams on hematology analyzers (neutrophils outside limit, neutrophils inferior deviation, neutrophils right deviation, eosinophils outside limit, confluent neutrophils and eosinophils, granulocytes outside inferior limit, two or more neutrophils coded groups or eosinophils groups, tendency of granulocytes to form one group, abnormal (grey) granulocytes color), and altered Random Distribution Width-RDW.[6]

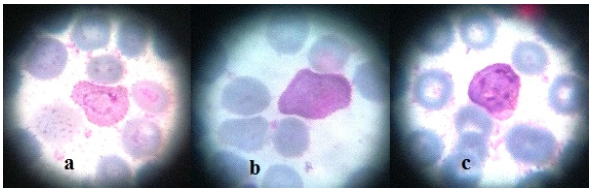

Fig-1: P vivax on smear shows trophozoite (a), gametocyte (b), and schiznot (c) stages

Methods

This is a retrospective, cross sectional, descriptive study. Seventy cases of Plasmodium vivax, positive on peripheral blood film, were included in this study. Sample with deranged LFTs and RFTs were excluded from the study.

Methods used to diagnose malaria involved peripheral film, both thick and thin blood smear. CBC was performed on Sysmex XE2100, Sysmex XE5000 instruments. These instruments use highly sophisticated technology using RF/DC Detection Method, Hydro Dynamic Focusing (DC Detection), Flow Cytometry Method Using Semiconductor Laser, and SLS-Hemoglobin Method.

Results

Majority patients i.e. 84.3% (n=59/70) showed thrombocytopenia with mean platelet count of 95.7 x109/L ranging from 18 to 333 x109/L. Pseudoeosinophilia was noted in 77.1% patients (n=54/70) with a gap of 0.8-25.1% between the analyzers' reported eosinophils and actual percentage on smear. Other findings are summarized in the following **table-1.**

WBC scatter-grams showed by hematology analyzers exhibit different patterns (eosinophils outside limit, merged dots of neutrophils and eosinophils, granulocytes outside inferior limit, equal to or more than two eosinophil coded areas, and abnormal (grey) granulocyte color)

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Table-1: MPV (Mean Platelet Volume), PDW-C (Platelet Distribution Width-Coefficient of Variation) MCV (Mean Corpuscular Volume of RBCs, RDW (Random Distribution Width-Coefficient of Variation of RBCs), WBCs (Total White Blood Cells). MPV and PDW were not reported by analyzers in 45 patients out of 70. RDW-CV was not calculated in only one patient.

Parameters	No. of Patients	Mean	Range
Platelets (x109/L)	70	95.7	18-333
MPV (fL)	45	10.6	8.6-13
PDW-CV (fL)	45	13.2	8.3-19.8
Hemoglobin (g/dL)	70	11.3	4.1-16
MCV (fL)	70	87.2	6-128
RDW-CV (fL)	69	18.1	13.83.1
WBCs (x109/L)	70	7.28	21-22.9
Monocytes (%)	70	10.1	1-30

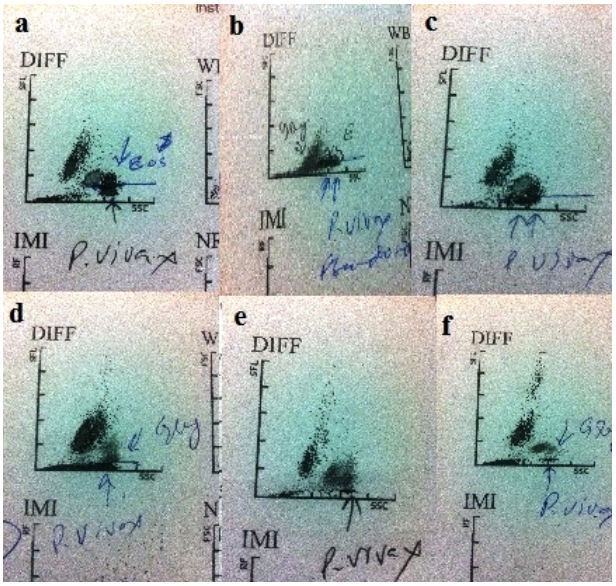

Fig-2: Eosinophils outside limit (a), equal to or more than two eosinophil coded areas (b), merged dots of neutrophils and eosinophils (c&e), granulocytes outside inferior limit (d), and abnormal (grey) granulocyte color (d&e).

Discussion

Malaria has been a major threat to human health for centuries in terms of morbidity and mortality. In the present study, thrombocytopenia was one of the leading hematological abnormality in 84.3% (n=59/70) patients with *P vivax* malaria.[7]

In this study, the patients with alteration in MPV and PDW were 12.7% (n=7) and 37.8% (n=17), respectively, out of 45 patients.[8] In 15 patients, these two parameters were not measurable. Considerable anemia (Hemoglobin <10 g/dL) was found in 32.8% (n=23) of the patients.[9] However, in our study, some patients showed microcytosis & hypochromia 22% (n=14) and macrocytosis 22% (n=14), favoring nutritional causes of anemia. A wide range of total WBCs was seen between 2.1-22.9 $x10^9$/L and mean of 7.3$x10^9$/L. Malaria could also be associated with Leukopenia (11%, n=8 in our study).[10] Pseudoeosinophilia is a striking finding on modern hematology analyzers. This is a phenomenon, in which eosinophils reported by analyzers are much different from actual eosinophils (both percentage and absolute number). In our work, 76.4% of the patients (n=52/68) showed pseudoeosinophilia. In two patients, this gap was not measureable. The gap between pseudo- and actual eosinophils ranged between 0.3 and 25.1 with a mean of 6.67%, in 52/68 patients. **A** gap of more than 5% between reported eosinophilia and actual eosinophils is significant.[11] In our work we found 51.4 % (n=35/68) of the total patients showed pseudo eosinophilia more than 5%. Monocytosis is attributed towards active phagocytosis of the parasite by monocytes (mean monocyte percentageage of 10.6% with a range of 1 to 30% with 42.8% (n=30) patients showing monocytosis in our work).[12]

Another finding in our record is Altered RDW with a mean of 18.1 N= 11-14 (range 13-38.1 fL).[13] In our study, WBC scatter-grams showed by hematology analyzers were (eosinophils outside limit, merged dots of neutrophils and eosinophils, granulocytes outside inferior limit, equal to or more than two eosinophil coded areas, and abnormal (grey) granulocyte color).[14]

Conclusion

To conclude: we suspect *P vivax* malaria when CBC, run on automated analyzers, gives information in the form of an integrated pattern of results. When thrombocytopenia along with raised MPV & PDW, anemia, leukopenia, monocytosis, eosinophilia (Pseudoeosinophilia in actual), abnormalities of WBC scatter-grams, Monocytosis and altered RDW are read in collaboration, gives a strong suspicion of *P vivax* infestation. Further diagnostic tests are to be run such as Thick and Thin Blood Smears, Immune Chromatography, PCR for DNA detection, Malarial LDH detection by Gel Agglutination for work up of malaria.

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References

1. Centers for Disease Control and Prevention. Malaria. Available at http://www.cdc.gov/malaria. Accessed Jan 20, 2015.
2. World Malaria Report 2014. WHO Global Malaria Programme Trends in infections, cases and deaths. Geneva, Switzerland: World Health Organization.
3. Mackintosh CL, Beeson JG, Marsh K. Clinical features and pathogenesis of severe malaria. Trends Parasitol. 2004 Dec; 20(12):597-603.
4. Elliott SR, Fowkes FJ, Richards JS, Reiling L, Drew DR, Beeson JG. Research priorities for the development and implementation of serological tools for malaria surveillance. F1000Prime Rep. 2014 Nov 4; 6: 100.
5. Beatrice A, Yolanda C, Francesco C and Donald T. Pathogenesis of Malaria in Tissue and Blood. Meditter J Hematol Infect Dis 2012, 4(1).
6. Ali HA, Abdulla MU, Nadeem JY, Ahmed SA, Dujana AH, Ahmed AS. Malaria and Hematological changes. Volume 24, April-June 2008 (Part-I) Number 2.
7. Alfonso J. Rodríguez M, Elia S, Miguel V, Carmelina P, Rosa C, Melissa A, and Carlos FP. Occurrence of Thrombocytopenia in Plasmodium vivax Malaria. Clin Infect Dis. (2005) 41 (1):130-131.
8. Fábio ALS, Soraya BRS, Natasha PC, Andréia FN, Thamires OGM, Eduardo RAJ and Cor JFF. Altered platelet indices as potential markers of severe and complicated malaria caused by Plasmodium vivax: a cross-sectional descriptive study. Malaria Journal 2013, 12: 462.
9. Claire LM, James GB, Kevin M. Clinical features and pathogenesis of severe malaria. Volume 20, Issue 12, December 2004, Pages 597603.
10. Alfonso J. Rodríguez M, Elia S, Miguel V, Carmelina P, Rosa C, Melissa A, and Carlos FP. Occurrence of Thrombocytopenia in Plasmodium vivax Malaria. Clin Infect Dis. (2005) 41 (1):130-131.
11. Huh HJ, Oh GY, Huh JW, Chae SL. Malaria detection with the Sysmex XE-2100 hematology analyzer using pseudoeosinophilia

and abnormal WBC scattergram. Ann Hematol. 2008 Sep; 87(9):755-9.
12. Zeeba SJ, Safia R, Mohammed JH, Farhat N, and Sujata J.. An Analysis of Hematological Parameters as a Diagnostic test for Malaria in Patients with Acute Ile Illness: An Institutional Experience. Oman Med J. Jan 2014; 29(1): 1217.
13. Ashis KS, Somnath M, Subhas CH. Comparison of Hematological Parameters between Plasmodium Falciparum, Plasmodium Vivax and Control Group. Int J Med Res Health Sci. 2014; 3 (1): 120-127.
14. Germán CZ, Thomas H, Martin PG. Automated hematology analysis to diagnose malaria. Malaria Journal 2010, 9:346.

Medical News

BENZODIAZEPINE OVERDOSE DEATH RATE 'HAS INCREASED FOUR-FOLD'

Benzodiazepines are a class of sedatives that includes Xanax, Valium and Klonopin. Although the Centers for Disease Control and Prevention have focused on opioids in the wake of the worsening drug epidemic in the US, results from a new study place benzodiazepines center stage in thisepidemic.

During the 18-year study period, the benzodiazepine overdose death rate increased four-fold, prompting researchers to call for interventions to reduce use.

Published in the American Journal of Public Health, the study was conducted by researchers at Albert Einstein College of Medicine and the Montefiore Health System in New York, as well as the Perelman School of Medicine at the University of Pennsylvania.

Patients are prescribed benzodiazepines for conditions such as anxiety, mood disorders and insomnia; in the US each year, an estimated 1 in 20 adults fill a prescription for a benzodiazepine.

These sedatives are considered a safe and effective treatment, but their long-term use can lead to addiction. Furthermore, there are certain side effects attached to them, including daytime drowsiness and a "hung-over feeling," increasing risk of automobile accidents.

They can also make breathing problems worse and can lead to falls in the elderly.

When used with alcohol, benzodiazepines can be dangerous, and overdoses can be serious.

In 2013, overdoses from the class of drugs made up 31% of the 23,000 prescription drug overdose deaths in the US. However, little was known about benzodiazepine prescribing trends or fatalities.

To further investigate, the researchers, led by Dr. Marcus Bachhuber, looked at data from 1996-2013, using the Medical Expenditure Panel Survey and multiple-cause-of-death data from the Centers for Disease Control and Prevention (CDC).

They found that the number of adults who filled a benzodiazepine prescription increased by 67% during the study period, which spanned 18 years; it went from 8.1 million prescriptions in 1996 to 13.5 million in 2013.

And for those adults who filled a prescription, the average quantity that was filled during each year more than doubled from 1996-2013.

Furthermore, the overdose rate increased four-fold, from 0.58 deaths per 100,000 adults in 1996 to 3.14 deaths per 100,000 adults in 2013.

"We found that the death rate from overdoses involving benzodiazepines, also known as 'benzos,' has increased more than four-fold since 1996 - a public health problem that has gone under the radar," says Dr. Bachhuber.

Senior study author Dr. Joanna Starrels says there may be two possible reasons for the increase in benzodiazepine deaths. Firstly, those "at risk for fatal overdose may be obtaining diverted benzodiazepines," meaning they are obtaining them from sources other than medical providers.

Another reason is that using benzodiazepines with alcohol or drugs puts people at greater risk for fatal overdoses. She says opioids are involved in 75% of overdose deaths that involve benzodiazepines.

Courtesy: medical news today