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ASSOCIATION BETWEEN ATYPICAL DEPOLARIZATION IN CELL-DYN 3200 AND THE PRESENCE OF PLASMODIUM SPP IN BLOOD IN Dr. SOETOMO HOSPITAL SURABAYA

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ABSTRACT

Background*: Malaria is a parasitic disease worldwide with a high morbidity and mortality. A rapid and accurate method is needed to detect the presence of malaria parasites in blood. A flagging system atypical depolarization (atypdep) in CBC results from Cell-Dyn 3200 has been related with malaria infection.* ***Materials and Methods****: An observational cross sectional approach with 48 samples obtained from inpatients of the Dr.Soetomo Hospital, Surabaya. Samples were screened by Cell-Dyn 3200 analyzer for atypdep flagging in CBC. Positive samples were later confirmed by microscope to detect malaria parasites.* ***Results****: From 48 samples with atypdep flagging, 7 samples were malaria positive on peripheral blood smear (13.1%). Most frequent atypdep flagging was seen in malignancy (18.7%), and approximately 54.6% of the samples were not accompanied by fever symptoms. Lekositosis and anemia each were found in 20 samples (41.6%) and thrombocytopenia in 33.3%.* ***Conclusion****: The presence of atypdep flagging in Cell-Dyn 3200 does not necessarily indicate the existence of malaria or it could be said that atypdep flagging is not always associated with presence of malaria infection. The usage of an atypdep flagging in non-endemic areas such as Surabaya is just an alert sign to evaluate malaria infection rather than a screening method to detect malaria.*

Key words: *Malaria, atypical depolarization, hematology analyzer*

INTRODUCTION

Until now, Malaria remains the most important parasitic disease worldwide and causes health problems especially for those living in endemic areas. Early diagnosis relies crucially on clinical suspicion. A clinician suspecting the disease has to request explicitly malaria examination by blood smears. Lack of clinical suspicion is a well-known factor for a missed diagnosis, which contributes substantially to patient morbidity and mortality in this disease.[1] Of the 300 – 500 million cases of malaria infection which are estimated to occur annually, approximately 2–3 million of these are fatal.[2] the high frequency of severe clinical complications and mortality in endemic regions is exacerbated by delayed or inefficient treatment, limited access to clinical and laboratory services and the increasing influence of drug resistance.[3,4]

The female anopheline mosquito transmits malaria parasites, and after infecting a new host, the parasites are carried in the blood to the liver where they undergo a hepatic stage of multiplication. After a period of 9 to 16 days, the parasites return to the bloodstream and infect red cells. The typical spiking fever of malaria occurs when the red cells rupture and release free parasites.[3]

Patients with symptoms of fever and malaise in nonendemic areas will usually consult a clinician. However, in many countries malaria ranks as a relatively infrequent cause of pyrexia and thus may not be considered as part of the differential diagnosis. This is especially true if a complete clinical/travel history is not obtained. In such situations, clinicians may only initially request general screening tests such as a full blood count (FBC).[3] while a diagnosis of malaria can be established by microscopic examination of thin and thick blood film[4], although the investigation does not necessarily indicate the existence of parasites.

Microscopic investigation of stained thick and thin blood smears has been the reference standard for malaria detection and species identification for decades. Recently, a number of alternative diagnostic approaches have evolved,

including detection of *Plasmodium* species DNA stained with acridine orange in a quantitative buffy coat analysis, PCR methods, and assays based on detection of circulating *Plasmodium* species specific antigens. Recent studies using automated hematology analyzers have demonstrated unexpected abnormalities in differential white blood cell plots and reticulocyte histograms from patients with malaria.[6]

In normal blood samples, the only depolarizing WBC events are eosinophils. With Cell-Dyn multiangle polarized scatter separation (MAPSS) analysis, normal eosinophils viewed in the polarized-90° versus depolarized-90° (NEU EOS) plot form a distinct cloud of events that are color coded green. The depolarization of these cells is due to a component of eosinophil granules (Figure 1).[3]

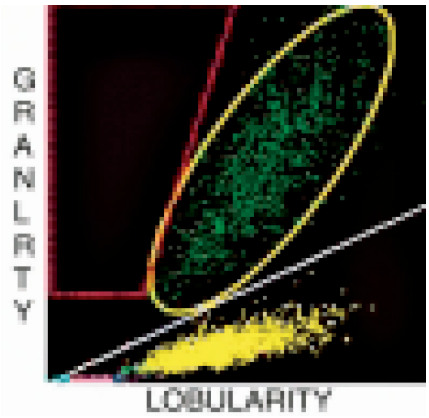

Figure 1. Granularity (90° depolarization axis) versus lobularity (90° polarization axis) plot showing typical Cell-Dyn 3200 eosinophil depolarization pattern. Normal eosinophils are located within the area demarcated by the yellow oval line, and the atypical depolarization region indicated by the red broken line does not normally contain any events.[3]

During the intraerythrocytic stage, a malaria parasite digests and breaks down hemoglobin to its constituent parts heme and globin. The globin is used as a protein source by the parasite and the heme is converted by an enzyme (heme polymerase) to hemozoin or malaria pigment. The parasite initiates this process because heme is toxic to the parasite whereas hemozoin is not. In contrast to nondepolarizing heme, hemozoin has a distinctive ability to depolarize light.

In the malaria parasite cell cycle, the malaria-infected red cells rupture at the schizont stage and the parasites are released together with hemozoin aggregates into the plasma. By an as-yet-unknown mechanism, circulating phagocytic WBCs (monocytes and neutrophils) then ingest the liberated free hemozoin. Consequently, normally nondepolarizing monocytes and neutrophils will depolarize light when they contain aggregates of hemozoin. this will cause appearance of abnormal dots on neu-eosin scatter plot.[3]

Research on detection of hemozoin by hematology analyzer has been done[1,2,3,4,5,6,7]. It was reported that the presence of one or more atypical depolarizing events can be attributed to malaria. Discovery of the abnormal depolarization pattern in patients with unknown fever should be considered to possibility of malaria infection, so microscopic examination by stained thick and thin blood smears as a confirmation needs to be done. A study in Portugal by Hanscheid et al[5] reported that diagnosing of malaria by detection of hemozoin using hematology analyzer obtain 95% sensitivity and a 88% specificity. While a South African study found a 72% sensitivity and 96% specificity.

In the Dr.Soetomo Hospital, Surabaya, atypical depolarizing events are often found in complete blood count results. Surabaya is not a malaria endemic area, but Dr. Soetomo Hospital is a referral hospital for the eastern Indonesian region, so that the patients are estimated to come from various regions. Most patients were examined with a diagnosis of other diseases, without any suspicion of malaria infection. Based on the fact, the researchers wanted to know whether the presence of atypical depolarization was actually due to malaria infection. If this was true, is it possible that existence of atypical depolarization (Atypdep) could be used a screening marker for malaria in non-endemic areas such as Surabaya? Is there any association between the presences of atypdep flagging with the plasmodium in the blood in non-endemic areas such as Surabaya?

MATERIALS AND METHODS

This research was done in the Laboratory of the Department of Clinical Pathology, Dr.Soetomo Hospital Surabaya during February to May 2010. Samples were obtained by selecting CBC results of inpatients in Dr. Soetomo Hospital. Samples of venous blood with EDTA anticoagulant were examined for CBC with a Cell-Dyn 3200 Hematology analyzer. CBC results showing atypdep flagging were included in this study. These samples were examined by thin blood smear examination with Giemsa staining to find and determine the types of parasites. Samples were considered positive when parasites were found in thin blood smears. The numbers of parasites were counted per 1000 erythrocytes, and samples were considered negative if in the 50 fields of emersion parasites were not found. Examination was conducted by 2 persons, a laboratory technician and a medical doctor. This study design was a descriptive observational study through cross-sectional approach, data and results were presented in the form of tables and figures.

RESULTS

During the period of the study 48 samples were obtained that fullfield the criteria (males 64.5%, females 35.5%)

with a variety of diagnosis. Most of the atypdep flagging was found in adult patients (60.4%) and also in 5 samples of neonates. Lekositosis and anemia were each found in 20 samples (41.6%), while thrombocytopenia was found in 16 samples (33.3%). Sample characteristics can be seen in Table 1. Of the 48 samples collected, only 7 samples were malaria positive with a thin smear examination, or about 13.1% only. Of the positive samples, almost all of them showed fever and a history of malaria endemic areas. Of malaria positive samples there were 5 samples showing anemia and thrombocytopenia (71.4%). Of all samples collected, atypdep appears most in the group of malignancy or tumor disease as much as 9 people, or 18.7%. (Table 2)

There are some patterns of atypical depolarizing events, some of which can be seen in figure 2a,2b,2c. Parasites were found among malaria positive patients in various phases (trophozoit, schizont, gametocytes).

Table 1. Samples characteristics

Parameter	number	Persentage (%)
Sex		
Male	31	64.5
Female	17	35.5
Age		
< 1 yr	5	10.4
1–< 18 yr	7	14.5
18–60 yr	29	60.4
> 60 yr	7	14.5
Hb level		
< 6 g/dl	-	
6–8 g/dl	4	8.3
> 8–11 g/dl	15	31.2
> 11–18 g/dl	28	58.3
> 18 g/dl	1	2.1
Temperature		
< 38°C	31	64.5
>= 38°C	17	35.5
Platelet level		
< 150,000	16	33.3
150,000–450,000	24	50
> 450,000	8	16.6
WBC		
< 4,000	4	8.3
4,000–11,000	24	50
> 11,000	20	41.6
Persentage of eosinophil		
<=7%	43	89.5
> 7%	5	10.5

Table 2. Clinical diagnosis of the positive atypdep patients

Diagnosis	No of sample	Persentage (%)
Trauma (Traffic accident)	5	10.5
Urinary bladder diverticle and chronic colitis	1	2
Nephrotic syndrome	1	2
Post partum	1	2
DHF	6	12.5
Malignancy and/tumor	9	18.7
Down syndrome	2	4.1
CKD	2	4.1
Hydrocephalus	1	2
Sepsis	3	6.2
BPH	1	2
Kidney stone	2	4.1
Suspected malaria	4	8.3
Combustio	3	6.2
Febris	2	4.1
DM	1	2
Decubitus ulcer	1	2
Pancytopenia	1	2
Hirschprung's disease	1	2
UTI	1	2
Hemolitic anemia	1	2

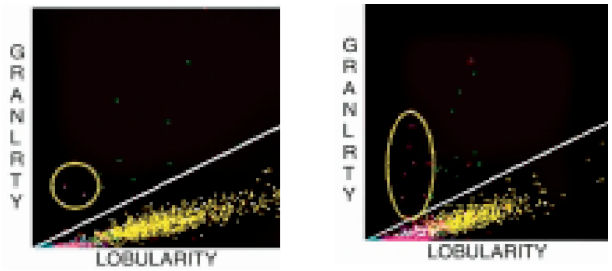

Figure 2a. Samples showing occasional atypical depolarizing purple events (within yellow broken boundaries).

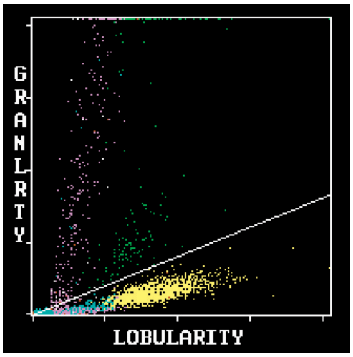

Figure 2b. Samples showing many atypical depolarizing purple events.

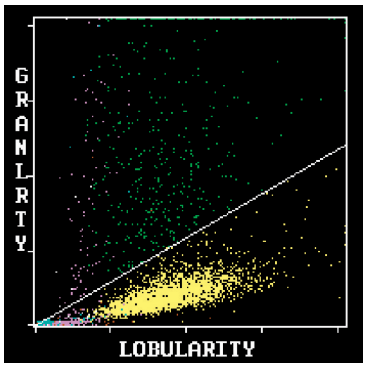

Figure. 2c. Malaria samples with mixture of abnormal depolarizing purple and green events that are not in the position normally associated with typical eosinophils

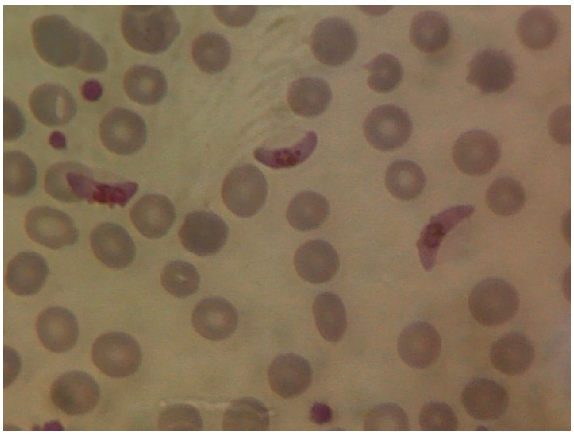

Figure 3. Trophozoit phase of plasmodium falcifarum (banana form)

DISCUSSION

In this study, out of 48 samples with atypep flagging, only 7 samples were malaria positive (13.1%). These results are not in accordance with various previous studies that have been done in several countries reporting that the sensitivity and specificity of atypical depolarization in detecting malaria is very high. This could be due to differences in sampling population In previous studies, samples taken from patients with clinical symptoms of malaria, and also done mostly in endemic malaria areas. While in this study, samples were taken at random, just based on the presence of atypdep flagging on CBC results regardless of clinical symptoms and diagnosis. After confirmation by thin smear examination, only 7 samples were positive for malaria. This shows that for non-endemic areas such as Surabaya (low prevalence), the appearance of atypdep is not yet certain in malaria infection .Therefore, it is important especially for patients with fever whose CBC results show atypdep flagging to confirm this by thick or thin smears in order to prove the existence of malaria infection.

In this study, percentage of atypdep flagging that appeared in diseases without fever and other symptoms of malaria was nearly 65% and many were shown in malignancies this proves that the presence of atypdep is not only caused by the presence of hemozoin or malaria pigment in monocytes or neutrophils, but there may be other causes such as small cell fractions that capable to depolarizing light in addition to eosinophils.

Changes of the parameters such as WBC, red blood cells and platelets in malaria patients are generally not specific. Some studies reported that the occurrence of thrombocytopenia in patients with clinical symptoms of malaria is an important indicator of malaria. Although the frequency of occurrence of thrombocytopenia reported was about 80%,[3,9] but these results varied in different studies that have been conducted. In this study, thrombocytopenia was found in 5 out of samples from the 7 malaria positive samples or approximately 71.4%. Similarly, anemia was found in 5 samples, while the number of WBC showed no characteristic changes .

Of the malaria positive samples, all were imported malaria. All positive samples did not come from endemic areas, however, there was a history of traveling to endemic areas. In this study, one sample showed a negative thin smear with a history of malaria therapy 1 week before. This is consistent with the theory that in patients who are in recovery where parasites can no longer be found in the blood, atypdep flagging can still occur because of atypical depolarizing clearance of malaria pigment is slow. In some individuals, this malaria pigment can remain in circulation until 3 weeks after recovery[3].

There is a reference reporting that pseudoeosinophilia is associated with the emergence of atypical depolarizing.[8] However, in this study, eosinophilia was found just in 5 samples or 10.4%. Also lekositosis as much as 20 samples (41.6%) raises a question, whether lekositosis may be related with the emergence of atypdep ? The emergence of atypdep in neonates as much as 5 samples also need to be considered, whether neonatal blood could influence the occurrence of atypdep flagging. A further study is needed to determine the factors that lead to the emergence of atypdep flagging, so that atypdep flagging is not merely focused on the presence of malaria, but other possible causes as well. However, when atypdep flagging is found, it is important to confirm this by blood smear examination for malaria.

Further studies are needed to determine the factors causing the emergence of atypdep flagging because in this study there are several limitations, among others:

- Detection of plasmodium is influenced by the quality of the staining and the skills and Expertise of examiners
- positive results are influenced by the prevalence
- More samples are needed.

CONCLUSION AND RECOMMENDATION

It was found that the rise of atypdep flagging does not always indicate a malaria infection or it could be said that atypdep flagging is not always associated with the occurrence

of malaria infection because from the 48 samples only 13.1% positive on blood smear. So the emergence of atypdep flagging on Cell-Dyn 3200 instrument can not be used as a screening of malaria in non-endemic areas such as Surabaya.

Additional criteria for non-endemic areas such as Surabaya are needed, when the existence of this atypdep flagging suspicious of malaria infection, for example: 1. Frequency of atypdep appearance in the same patient 2. Existence of thrombocytopenia 3. Presence of clinical symptoms (fever, chills, etc.). This needs to be done by scoring and with a larger number of samples. Moreover, further studies should be conducted to identify other factors leading to the emergence of atypdep flagging.

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