RDT accuracy based on age group in hypoendemic malaria

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Abstract. Malaria is still one of the problem of community health in Sumatera. This study was carried out to compare RDT accuracy in some groups of age in hypoendemic malaria. The microscopy test was investigated by 3% Giemsa Staining and examined by a trained laboratory technician. RDT was carried out by using Monotes Test Drive. The accuracy of RDT diagnostic was commonly significant in all groups of age, excepting the group of age > 65 years old (p=0.393). The highest sensitivity of RDT was commonly in group of age ≤ 5 years old and decreased in the older group of age. Otherwise, the lowest specificity was found in group of age ≤ 5 years old and the highest in group of age 6-15 years old. The highest PPV and NPV was found in group of age 16-65 years old and ≤ 5 years old, respectively. The highest of parasite density was found in a group of age ≤ 5 years old (644.4±494.5 parasite/µl) and the lowest in group of age > 65 years (400±490.7 parasite/µl). The accurate diagnosis of RDT reduces by increasing of age.

1. Introduction

Malaria is a parasitic disease that is still as a major health problem in the world. It is estimated more than 3.2 billion population in the world have some risk factors for the suffering of malaria. WHO reported in 2015, there are 214 million new cases of malaria and more than 438000 deaths are due to malaria\(^1\). Baby, toddler and pregnant women are at the high risk of suffering of malaria\(^2\).

Morbidity and mortality rate of malaria commonly fluctuates. Increasing of case number is due to late diagnosis and resistance to antimalarial drugs. The late diagnosis is influenced by the availability of diagnostic test in that area. The specific clinical manifestation in every endemic area can be applied as a step in the screening of malaria, cannot be applied as a definitive diagnosis\(^3\). The microscopic test is still golden standard in the diagnosis of malaria. Less of trained microscopic laboratory technician is one of the main problems of malaria diagnosis.

Rapid Diagnostic Test (RDT) is one of alternative diagnosis commonly used in an area without a trained microscopy laboratory technician. The principal of RDT is to detect Plasmodium antigen by using immunochromatographic methods in a dipstick form.

RDT accusation is still various in some areas. Many factors are contributed, such as parasite density in blood and instruments damage. Immune body system (antiparasite system) is one of some factors contribute to parasite density that is commonly influenced by the age. Otherwise, RDT damage commonly due to its storage temperature is higher than it is advised.

The aim of the present study was to compare accuracy diagnosis of RDT to a peripheral blood test by using a microscope as the golden standard, in the hypoendemic area. The accuracy of those RDT will compare in some groups of age.
2. Methods
This study was part of research in order to design a diagnosis model for asymptomatic malaria patient in the hypoendemic area. This study was conducted from March 2015 to December 2018.

The present study is a longitudinal study that observed each subject for 2 weeks. Each of the subjects was observed in only two periods of suffering malaria as long as the research. The sample was taken from 4 primary health cares and 25 villages in Batubara Regency which is hypoendemic malaria in Province of Sumatera Utara.

Each sample that involved in this study examined by RDT and microscopic test by 3% Giemsa Staining as the golden standard for malaria diagnosis. The microscopic test was investigated by at least two trained laboratory technician. The temperature at the axilla of the subject determined as fever if $\geq 37.5^\circ C$. Another characteristic was taken from questionnaire and observation. RDT that was used was Monotest Test Device, Malaria Pf/Pv Ag Rapid Test (whole blood) with 100% of sensitivity and 98.7% of specificity.

3. Result
This study was followed by 2173 population stayed permanently in the study area. Fever was still the main symptom of malaria, but 45.3% samples did not have the symptom. The most group of age and gender were found in 16-65 years old and male, respectively (table 1).

Table 1. Characteristic of the sample.

| Characteristic | n  | %  |
|----------------|----|----|
| Ages           |    |    |
| ≤ 5 years      | 117| 5.4|
| 6-15 years     | 971| 44.7|
| 16-65 years    | 1064| 49.0|
| > 65 years     | 21 | 0.9|
| Gender         |    |    |
| Male           | 979| 45.1|
| Female         | 1194| 54.9|
| Sign/Symptom   |    |    |
| Fever (+)      | 1189| 54.7|
| Fever (-)      | 984 | 45.3|

In this study 671 subject was found suffering from malaria, 53% had afever as their symptom (clinical malaria). About 32.5% subject with malaria came without fever symptom classified as asymptomatic malaria. Even 14% of the subject diagnosed as submicroscopic malaria after passing serial microscopy test. Plasmodium vivax was the most causes of malaria in this research (table 2).

Table 2. Characteristic of malaria patients.

| Characteristic | n  | %  |
|----------------|----|----|
| Type of diagnostic |    |    |
| Clinical malaria  | 359| 53.5|
| Asymptomatic malaria | 218| 32.5|
| Submicroscopic malaria | 94 | 14.0|
| Etiology        |    |    |
| Plasmodium vivax | 427| 63.6|
| Plasmodium falciparum | 91 | 13.6|
| Mixed           | 153| 22.8|
In general, clinical malaria is the most case found in all groups of age. Asymptomatic malaria and submicroscopic malaria were the most cases found in a group of age 16-65 years old followed by a group of age 6-15 years old (table 3).

| Type               | ≤ 5 years |       | 6-15 years |       | 16-65 years |       | > 65 years |
|--------------------|-----------|-------|------------|-------|-------------|-------|-----------|
|                    | n   | %          | n   | %          | n   | %          | n   | %          |
| Clinical malaria   | 25  | 7.0        | 135 | 37.6       | 198 | 55.2       | 1   | 0.3        |
| Asymptomatic malaria| 10  | 4.6        | 90  | 41.3       | 116 | 53.2       | 2   | 0.9        |
| Submicroscopic malaria | 10  | 10.6      | 33  | 35.1       | 49  | 52.1       | 2   | 2.1        |

The highest parasite density was found in group of age ≤ 5 years old (table 4).

| Parasite density | ≤ 5 years |       | 6-15 years |       | 16-65 years |       | > 65 years |
|------------------|-----------|-------|------------|-------|-------------|-------|-----------|
|                   | Mean      |       | Mean       |       | Mean        |       | Mean      |
|                   | 644.4     |       | 619.15     |       | 641.6       |       | 400       |
|                   | 494.50    |       | 425.95     |       | 442,768     |       | 490.71    |

In general, RDT test was significant in all groups of age, except in the group of age > 65 years old (p=0.393). The highest sensitivity was found in a group of age ≤ 5 years old and reduced by increasing of age. Otherwise, the lowest specificity was found in a group of age ≤ 5 years old and the highest in a group of age 6-15 years old, respectively. The highest PPV and NPV were found in a group of age 16-65 years old and ≤ 5 years old, respectively (table 5).

| Type               | ≤ 5 years |       | 6-15 years |       | 16-65 years |       | > 65 years |
|--------------------|-----------|-------|------------|-------|-------------|-------|-----------|
|                    | RDT (+)   | p value | RDT (-)   | p value | RDT (+)   | p value | RDT (-)   | p value |
| Malaria (+)        | 17        | 0.000  | 175       | 0.000  | 266       | 0.000  | 3          | 0.000  |
| Malaria (-)        | 0         | 0.000  | 72        | 0.000  | 194       | 0.000  | 2          | 0.000  |
|                   | Sens      | 100.01%| Spec      | 11.87% | Sens      | 100.0%| Spec      | 11.87% |
|                   | PPV       | 37.8%  | NPV       | 97.3%  | PPV       | 73.3%  | NPV       | 60.0%  |
|                   | NPV       | 100.0% | NPV       | 100.0% | NPV       | 100.0%| NPV       | 100.0% |

4. Discussion
Not all the primary health care in Batubara Regency have trained microscopic. Therefore the alternative choice is Rapid Diagnostic Test (RDT). Rapid Diagnostic Test (RDT) is malaria diagnosis test commonly used in malaria area that cannot do the microscopic test.

Many asymptomatic and submicroscopic malaria patients, showed that malaria diagnosis is not enough only by the symptom. Especially in group of age above 5 years old. This is related to parasite density. In general, the more ages the more the body’s immune system can suppress the density of parasites, thus affecting its clinical manifestations. Even need aserial test for those whom suspected as malaria patient with many risk factors. Therefore, malaria screening is the early detection of asymptomatic and submicroscopic malaria for malaria patient without fever symptom and serial test.

Otherwise, many malaria patients who are caused by Plasmodium vivax looked similarly with its clinical manifestation which relatively mild compared to Plasmodium falciparum. In general, these clinical manifestations are almost identical to many other areas.

Sensitivity and specificity of RDT compared to microscopy test are very various, but it is still good. Meenaa (2009) in his study determined sensitivity and specificity of RDT as 92.9% and 94.8%, respectively. Accuracy in the study relatively higher due to the study sample was malaria patients whom ambulatory at the hospital, that commonly had high parasite density. This study determined that RDT
accuracy varies in a different group of age. The sensitivity of RDT varies from 25% to 100%. Similar to specificity that varies from 72% to 89.3%. Commonly, the sensitivity of RDT is high in the young group of age and reduce in the older group of age. However, the opposite occurs in the specificity.

The result of this study, commonly similar to the previous study conducted in endemic malaria, RDT has low sensitivity although its specificity is good enough\textsuperscript{6,7}. VanderJagt found the sensitivity of RDT less than 50%\textsuperscript{8}. This study determined the high of the accuracy of diagnosis is related to parasite density, the highest parasite density was found in a group of age \( \leq 5 \) years old. The similar result also found in another research\textsuperscript{9,10}.

Besides parasite density and \textit{Plasmodium} species that investigated\textsuperscript{11}, accurate diagnosis of RDT also influenced by the quality of RDT and procedural test. Whitty (2009) and Penhalbel (2005) in their study determined that there was a correlation accuracy diagnosis of RDT with its storage temperature\textsuperscript{12,15}. Accuracy diagnosis of RDT decreases if its storage temperature is higher than advised temperature. Moreover, its sensitivity can reduce an estimated 60%\textsuperscript{14}.

Although the accuracy is not similar to microscopic test, RDT as malaria alternative test is still necessary\textsuperscript{15}. Moreover, RDT also uses to detect asymptomatic malaria in hypoendemic area\textsuperscript{16}. Quality of RDT and procedures for using the tool correctly is very necessary to reduce its weakness, so that early detection of malaria can be conducted in the primary health care.

5. Conclusion
The sensitivity of RDT decreases with an increase of age. It is influenced by parasite density that related to the immune system (antiparasite disease) which is better by increasing of age. Asymptomatic malaria and submicroscopic malaria detection can conduct by investigate malaria suspected patient even though without fever symptom and also by serial test if the test is still negative on the first test. RDT is still can be used as an alternative diagnosis of malaria, especially in areas that have not microscopic laboratory technician.

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