EFFECT OF ARTIFICIAL INTELLIGENCE-BASED TECHNOLOGY IN MALARIA DIAGNOSIS

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ABSTRACT

BACKGROUND
Malaria diagnosis remains a key issue in remote area. The gold standard microscopy has limitations of its own and the commonly used rapid diagnostic tests have variable results. An artificial intelligence based digital cytometry platform was assessed for its comparison with microscopy and rapid diagnostics test in terms of accuracy and feasibility.

METHODS
100 patients with complaints of fever with chills were included in the study. They were screened for malaria infection by microscopy of thin peripheral smear, antigen based rapid diagnostic test and digital cytometry.

RESULTS
At higher malaria parasite concentration, the results between microscopy and digital cytometry were matching exactly. But at lower parasite concentration microscopy had lower sensitivity than digital cytometry. When compared to rapid diagnostic tests the digital cytometry had advantage of better species identification and better sensitivity.

CONCLUSIONS
In our study digital cytometry has greater sensitivity at lower parasite concentration as compared to rapid diagnostic test and microscopy. It was more accurate in species identification as compared to rapid diagnostic test.

KEYWORDS
Malaria, Malaria Diagnosis, Parasight, Digital Cytometry, Artificial Intelligence.


BACKGROUND
World malaria report 2018 estimates 219 million cases of malaria in 2017 and 4,35,000 deaths around the globe. Situation in a tropical country like India is no less worse with about 9.5 million cases of malaria reported in 2017 and about 1.25 billion which is 94% of Indian population are at risk of contracting Malaria. The key strategy for malaria management in India is to detect early and give prompt treatment. Many times malaria presents with vague clinical symptoms rendering it difficult to diagnose clinically. In such situations definitive diagnosis of malaria becomes very important for effective management. The situation becomes even worse in peripheral and remote areas where quality laboratory diagnosis facilities are lacking. The most common methods used for diagnosis is microscopy and histidine rich protein-2 and pLDH based rapid detection methods. As of today microscopy is the gold standard for diagnosing malaria but it requires qualified personnel and reports are subjective apart from being time consuming which may lead to fatigue in case of high test volumes. Rapid diagnostic tests are very good options in remote areas for diagnosis but they have their own disadvantages like persistence of HRP-2 in blood, low sensitivity at initial stages of lower parasitaemia and inability to give quantitative results and results very significantly across brands and batches. So, overall misdiagnosis remains a challenge in curbing the antimalarial resistance due to unnecessary prescriptions and preventing the morbidity and mortality secondary to missed malaria detection. Recent advances in molecular diagnostics like PCR and LAMP have shown very good results in accurate diagnosis but they are time consuming, costly and not feasible at all places. The “Parasight” digital cytometry based scanning and analysing device is combination of microscopy and automation based on artificial intelligence. It promises advantage of the gold standard microscopy while removing the disadvantages of microscopy like poor slide preparation, improper staining, fatigue and incomplete scanning of slide.

METHODS
Paediatric patients presenting with high fever with chills at clinic were informed about the study and consent was taken. 3 ml of EDTA sample was taken for the malaria study. Subjects were included in the study in the following manner - 50 patients who were detected malaria positive on peripheral slide or rapid diagnostic test were kept in group A. 50 patients who were negative in both microscopy and rapid diagnostic tests were kept in group B. The routine
method of malaria detection Giemsia stained peripheral smear (gold standard) and commercially available antigen based rapid diagnostic kit was used. Treatment was given on the basis of these routine methods. Along with this 5 µl of EDTA blood was used for malaria detection on the "Parasight" digital cytometry based scanning and analysing device. 5 µl of blood was stained using the company provided staining solution and buffer. This stained solution was pipetted into a flow cell disposable cartridge which prepare a uniform monolayer of blood cells. On the basis of internal calibration parameter, the computer vision image processing and statistical models rapidly identifies red blood cells, white blood cells, platelets and malaria parasites. The result was given in the form of species of malaria and % of RBCs infected. The final data was analysed using statistical software SPSS v20.0.

RESULTS

Microscopy vs. Digital Cytometry
Out of 28 cases detected as P. vivax on microscopy, 100% subjects were detected as P. vivax on digital cytometry as well. Similarly, out of 10 P. falciparum and 12 mixed infection on microscopy all subjects were detected as falciparum and mixed infection respectively. However out of 50 microscopically negative malaria subjects 3 were detected as P. falciparum and 5 were detected as P. vivax. The mean parasite concentration on digital cytometry in 50 microscopically positive subjects was 0.0515% and in 8 subjects which were positive in digital cytometry and negative on microscopy the mean parasite concentration was 0.004%. The difference in mean parasite concentration was statistically significant. The p-value was 0.003.

Rapid Diagnostic Tests (RDT) vs. Digital Cytometry
Out of 23 cases detected as P. vivax on RDT, 21 (91.3%) subjects were detected as P. vivax on digital cytometry as well and 2 (8.7%) were detected as having mixed infection of falciparum and vivax. Similarly, out of 9 P. falciparum cases, 8 (88.8%) cases were detected as falciparum and 1 (11.2%) was detected as mixed infection and 6 mixed infection on RDT were detected as mixed infection in digital cytometry as well. However, out of 62 RDT negative malaria subjects 5 (8.1%) were detected as P. falciparum, 12 (19.3%) were detected as P. vivax and 3 (4.8%) were detected as mixed infection in digital cytometry. The mean parasite concentration on digital cytometry in 38 RDT positive subjects was 0.0562% and in 20 subjects which were positive in digital cytometry and negative on RDT the mean parasite concentration was 0.0236%. The difference in mean parasite concentration was statistically significant. The p-value was 0.005.

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>Digital Cytometry</th>
<th>Total</th>
<th>Mixed</th>
<th>Negative</th>
<th>pf</th>
<th>pv</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mixed</td>
<td>Negative</td>
<td>pf</td>
<td>pv</td>
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</tr>
<tr>
<td>RDT</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
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<td>42</td>
<td>5</td>
<td>12</td>
<td>6</td>
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<tr>
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<td>0</td>
<td>8</td>
<td>0</td>
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<tr>
<td>pv</td>
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<td>0</td>
<td>0</td>
<td>28</td>
<td>28</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>42</td>
<td>13</td>
<td>33</td>
<td>33</td>
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<td>100</td>
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</tbody>
</table>

Table 1. Comparison of Parasite Concentration Between Microscopy and Digital Cytometry

DISCUSSION
As WHO plans for elimination of malaria and prevention of antimalarial resistance, demand for accurate, rapid and cost-effective malaria diagnostic modality remains a desirable need. Various studies have shown results of gold standard microscopy and RDT below expectation in practical scenarios. The reasons for this below par results are numerous.

Table 2. Comparison of Species Identification Between RDT and Digital Cytometry

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>Mean ± SD Parasite Concentration (% RBC Infected)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive on Both Microscopy and Digital Cytometry</td>
<td>50 0.0515 ± 0.042</td>
<td>0.003</td>
</tr>
<tr>
<td>Negative on Microscopy and Positive on Digital Cytometry</td>
<td>8 0.0040 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>Positive on Both RDT and Digital Cytometry</td>
<td>38 0.0562 ± 0.044</td>
<td>0.005</td>
</tr>
<tr>
<td>Negative on RDT and Positive on Digital Cytometry</td>
<td>20 0.0236 ± 0.029</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Comparison of Parasite Concentration Between RDT and Digital Cytometry

Table 4. Comparison of Species Identification Between Microscopy and Digital Cytometry

The Parasight digital cytometry-based scanning and detection system for malaria has shown promising results in previous studies.\textsuperscript{10,11} We tested it for practical feasibility in terms of accuracy, cost effectiveness and rapidity. Our study has shown that in comparison to gold standard microscopy, the digital cytometry gave similar results (in terms of accurate species detection as well) when parasite concentration was on higher side (mean 0.0515\%). While at lower parasite concentration (mean 0.004\%) the routine microscopy in practical environment gave negative results. The percentage of such false negative on microscopy was 16\% as compared to digital cytometry. When the results were compared between RDT and the digital cytometry, the results were lesser matched as compared to microscopy. Almost 33\% cases detected as negative on RDT were diagnosed have malaria infection of different types in digital cytometry and nearly 10\% had mismatched species identification. Species identification was 100\% match in microscopy and digital cytometry comparison. In previous studies by Eshel Y et al 2017 done at India and African region, thin-smear microscopy was performed and parasitaemia levels were compared to values obtained from the device. At Apollo Hospital India, the device showed a Pearson correlation coefficient of 0.84, compared to microscopy (correlation coefficients of 0.98 for P. falciparum and 0.83 for P. vivax); at AKUH, the device showed a Pearson correlation coefficient of 0.85, compared to microscopy (correlation coefficients of 0.86 for P. falciparum and 0.55 for P. vivax).\textsuperscript{10} In a study by Srivastava et al 2015 for comparison of parasite species detection by PCR and its comparison with digital cytometry. From samples found to be positive by both PCR and SightDx, SightDx speculated 73.3\% of PCR P. falciparum + samples and 91.4\% of PCR P. vivax + samples, as shown in Table 3. Notably, all of the mixed infection samples were positively detected by SightDx as infected. Eleven mixed infections were reported as P. vivax, two were reported as P. falciparum, while one was reported positive. At the time of publication device didn’t had the algorithms to identify mixed infection.\textsuperscript{11} Since then the data has accumulated and the sensitivity and specificity has improved in terms of detection of mixed infections. It is very important to note that all three modalities – microscopy, digital cytometry and RDT usage was done in practical scenarios. We may see some improvement in highly standardised environment but then the purpose of this study was to conduct results in a practical environment and see the feasibility. The previous studies done in highly standardised environment have also shown the similar results in favour of digital cytometry as well. In normal laboratory setting the sensitivity of microscopy is estimated at around 500 parasites/μl\textsuperscript{12} and in our study the lowest parasite detection in digital cytometry observed was 25 parasite/μl. However, the limitations of the devices are that it requires robust dust and vibration free environment along with stable electricity. At higher RBCs concentration (>6.5 million/mm\(^3\)) the monolayer formation in flow cell cartridge is improper and the tests results gets rejected, which requires a repeat test with greater dilution in dye solution. The minimum of 5 sample needs to be processed at a time because of the design of the flow cell cartridge. Meticulous handling to avoid finger prints and scratch and dust free storage of cartridge is must to avoid failed scanning. The scanning errors may occur due to overfilling or under filling of flow cell cartridge, dust particles on cartridge, too much blood taken or high RBC count of sample, agglutinated sample. Scanning errors are flagged by the machine with probable cause of scanning error so it can be corrected appropriately. Overall, it is a very good platform as convenient, accurate and cost-effective alternative to microscopy and certainly a better option than RDT wherever device installation is feasible.

**CONCLUSIONS**

The digital cytometry and algorithm-based malaria scanning device Parasight may be used for malaria diagnosis as it provides similar and to an extent better results than gold standard microscopy and certainly it has advantages of reduced subjectivity and quantification of parasites. Studies with higher number of subjects and the confirmation of malaria infection at lower parasite concentration should be done by molecular based technology before it can be used widely across public platforms.

**REFERENCES**
