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Sensitivity and Specificity of Rapid Biosensors for Diagnosis of Malaria in Kenya: Have they Impacted the Disease Burden? A Perspective Study

Wambani Josephine[*1], Walekhwa Michael[2], Otieno Filex[3], Suge Titus[4]

[1]*Kenya Medical Research Institute (KEMRI) HIV Laboratory-Alupe, Kenya*

[2]*School of Medicine and Health Sciences, Kabarak University, Kenya*

[3,4]*School of Pharmacy, Kabarak University, Kenya*

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Abstract

Globally, about 3 billion individuals are at risk of contracting malaria every year, with over 400,000 deaths occurring in the Sub-Saharan Africa. The disease is caused by parasites of the genus plasmodium. Currently, six species of plasmodium have been implicated with Plasmodium falciparum bearing the greatest fatalities and burden. Globally, malaria cases are on the rise and with the emerging resistance to antimalarials, rapid and accurate diagnosis is of utmost importance. This review focuses on specificity and sensitivity of different biosensors for malaria diagnosis using several surface modification strategies. Biosensors and immunosensors offer superior analytical performance compared to other malaria tests. They target specific proteins within the parasite and in doing so; not only detects the disease but also could indicate the stage of the parasite. These biomarkers include *Plasmodium falciparum* histidine-rich protein 2, parasite lactate dehydrogenase, aldolase, glutamate dehydrogenase and biocrystal hemozoin. Biomarker based immunosensors and biosensors are efficient, low labor intensive, affordable, accurate and reliable. However, the main problem associated with mass utilization are based on biomarker characteristics: *PfHRP-2* persists longer in human tissue and blood circulation even if parasites are cleared, while aldose-based biosensors have low sensitivity. Currently due to such pitfalls, biosensors and immunosensors remain comparatively similar in overall performance compared to other diagnostic platforms such as rapid diagnostic tests. As such, with proper design and precision, biosensors and immunosensors could offer a promising future in scaling down malaria fatalities and burden.

KEYWORDS:

Plasmodium, biosensors, immunosensors, malaria, PfHRP-2, pLDH, aldose, hemozoin

I. INTRODUCTION

Malaria has over time constituted a significant public health threat across the world. While the disease affects persons living in all countries, those living in Low and Low Middle Income Countries (LMICs) represent the greatest burden (Krampa et al., 2017; Sultana et al., 2017). Over 80% of the cases of malaria worldwide occur in Africa, and especially among children (Ogony et al., 2019). Six species of the genius Plasmodium namely, *Plasmodium falciparum, Plasmodium malariae, Plasmodium knowlesi, Plasmodium ovale (P. ovale curtisi* and *P.ovale wallikeri), Plasmodium cynomolgi* and *Plasmodium vivax* are known to cause infection in humans (Krampa et al., 2017; Jain et al., 2014). However, greatest disease burden and fatalities are caused by *Plasmodium falciparum* (Ogony et al., 2019). Globally, about 3 billion individuals are at risk of contracting malaria disease every year, with over 400,000 deaths occurring in the Sub-Saharan Africa (Okoyo et al., 2021; Fornace et al., 2021; Ryan et al., 2020; Ogony et al., 2019). Based on the World Malaria Report of 2017, there were 216 million cases of malaria infection in 2016 up from 211 million cases in 2015 with estimated deaths of 445,000 in 2016 (Okoyo et al., 2021). According to the World Health Organization (WHO) data of 2018, about 228 million cases of malaria and 405,000 deaths were reported worldwide, with Africa representing the greatest number of cases and the highest mortality (Hussein et al., 2020).

In Kenya, malaria remains a major public health problem and cause of high morbidity and mortality with over 70% of its population being at risk of the infection (Okoyo et al., 2021; Watts et al., 2021). Annually, there are an estimated 3.5 million cases diagnosed and 10,700 deaths from malaria with the highest mortality rates in Western Kenya followed by the Coastal region (Watts et al., 2021; Touray et al., 2020; Desai et al., 2020; Ng'ang'a et al., 2020). More often, febrile patients are not diagnosed using laboratory tests, but rather based on clinical manifestation. Such patients end up taking antimalarials and antibiotics resulting in increased risk of drug resistance (Van Duijn et al., 2021). It is paramount for testing to be carried out real time, as it permits improved targeting of medicines to patients who have malaria, thereby reducing wastage and exposure of patients to drugs they do not need. Testing also provides a more accurate picture of the disease burden (Gachugia et al., 2020). For WHO set goals regarding elimination of Malaria by 2030 to be achieved, there is need for accurate and timely diagnosis and appropriate treatment of the disease (Boakye et al., 2021; De Freitas Borges et al., 2020).

Currently, different diagnostic approaches are available including microscopy, rapid diagnostic tests (RDTs), and nucleic acid amplification tests (Gachugia et al., 2020). These technologies have significant gaps and successes which may hinder or spearhead malaria eradication in Kenya. Use of these technologies is influenced by various factors including cost, simplicity, accessibility and availability of qualified personnel to perform the tasks (Gachugia et al., 2020). Desirable characteristics for diagnostic tests vary depending on the epidemiology, infrastructure and systems available, and goals of testing (Gachugia et al., 2020). Portability, ease of use, and robustness under adverse environmental are key characteristics of an ideal diagnostic tool if timely patient management is to be realized. Furthermore, affordability, shorter turnaround time, specificity and sensitivity is essential.

For RDTs their performance is affected by several factors including; parasite density, patient anti-malarial treatment history, pfhrp2/3 deletions, storage conditions and operator proficiency (Gachugia et al., 2020). Significant alteration of these factors results in erroneous results, poor management of patients, irrational use of anti-malarial drugs and inaccurate surveillance data (Gachugia et al., 2020). The challenges with the RDT include likelihood of false positives and false negatives due to the prolonged clearance time for histidine-rich protein 2 (HRP2) and HRP2 gene deletion respectively (Gachugia et al., 2020; Yamamoto et al., 2020). Moreover, RDT sensitivity also decreases with low parasitemia, genetic variability, and prozone effect. However, RDTs are easy to use, rapid, and affordable.

On the other hand, microscopic examination of Giemsa-stained blood smears is heavily utilized since it's the gold standard in malaria diagnosis. It is cost friendly though the entire process is labor-intensive and requires technical expertise for accurate diagnosis (Gachugia et al., 2020; Yamamoto et al., 2020). Gaps

in microscopy include shortage of trained personnel, lack of well-maintained microscopes and quality reagents, mis-identification or poor diagnosis of trophozoites, high workloads and poor performance in species identification and reporting compromising the quality of results (Gachugia et al., 2020). While improvements are possible, there is need for consistent financial and human resource investments, which are often lacking. Furthermore, microscopy requires an expensive diagnostic quality microscope, electricity, and routine replenishment of reagents. As a result, microscopy may not be ideal and in such cases RDTs have dramatically improved the diagnostic capability (Gimenez et al., 2021).

Interestingly nucleic acid amplification tests (NAATs) including PCR, LAMP, QT-NASBA, and ELISA are highly sensitive and are able to detect low density malaria infections. However, these methods are complex, prohibitively expensive, time consuming and require highly specialized reagents and facilities which cannot be easily used by the non-specialist in remote underdeveloped areas (Gachugia et al., 2020; Wu et al., 2020).

Finally, biosensors are very crucial in malaria diagnosis due to their superior analytical performance as compared to the conventional laboratory methods (Gimenez et al., 2021). Biosensors target specific biomarkers including *Plasmodium falciparum* histidine-rich protein 2 (PfHRP-2), parasite lactate dehydrogenase (pLDH), aldolase, glutamate dehydrogenase (GDH), and the biocrystal hemozoin (Yamamoto et al., 2020; De Freitas Borges et al., 2020; Krampa et al., 2017). Biosensors leverage biochemical interactions between a biological element attached to a transducer substrate and an analyte of interest from a certain bulk solution. This reaction leads to the alteration of a transduction property of the sensor (e.g. conductivity, temperature, absorbance, etc.) and this signal variation is proportional to the analyte concentration (De Freitas Borges et al., 2020; Dutta, 2020). The technology has capability of detecting asymptomatic individuals hence impacting on transmission dynamics, malaria control and possibly disease eradication. Biosensors come with a lot of advantages including relatively lower cost, lower time-to-perform, possibility of miniaturization and low limit of detection (LOD) (De Freitas Borges et al., 2020). Are fully automated and portable and hence can be used in local health facilities lacking basic infrastructure. Results are generated within a shorter timeframe hence decisions can be made promptly. Fully automation of the equipment guarantees the accuracy, reliability and reproducibility of the generated results (Yamamoto et al., 2020).

This review focuses on specificity and sensitivity of different sensitive biosensors for malaria diagnosis using several surface modification strategies. Summary of the biomarkers targeted during the course of malaria are also highlighted. The prevailing challenges and future outlook of the use of these technologies in the field are synthesized.

II. DISCUSSION

Biosensors and immunosensors have experienced unprecedented growth in recent years and seem to be the most promising sensing tools with several analytical benefits and cost efficiency (Turner, 2013; Mascini, 2007). This growth has been driven in part by the surge in demand for POC devices in clinical diagnosis where biological sensing is integrated with microelectronics to form portable analytical devices. To date, nearly sixty years after the first biosensor for glucose detection, the technology has been widespread in several fields of analyte detection (Mascini, 2007). Glucometers have evolved enormously, receiving vast commercial success (Lee, 2008). Among the types of sensors, electrochemical biosensors have received considerable interest in clinical diagnostics owing to key advantages in their design, assay simplicity, and superior analytical performance over conventional laboratory methods (Belluzo et al., 2008). These qualities make them suitable for POC application amidst efforts to improve and miniaturize electrochemical systems for portable devices (Belluzo et al., 2008).

Most attempts to create miniaturized electrochemical devices for on-site analysis have applied screen-printed electrodes (SPE) as transducers and various nanomaterials as signal amplification strategies

to improve the assay sensitivity (Solanki et al., 2011). Electrochemical immunosensors have been commonly applied to malaria diagnostic research given the benefits of low detection limits, wide linear response range, stability and reproducibility.

The choice of PfHRP-2 and LDH is still predominant, similar to RDTs. However, there is an increase in preference for pLDH possibly due to the persistence of PfHRP-2 antigenemia for several weeks after parasite clearance and reports of mutant strains from Africa and Asia with deleted PfHRP-2 gene (Bharti et al., 2016; Koita et al., 2012). Specificity and sensitivity of different biosensors for malaria diagnosis using several surface modifications strategies are well illustrated in this article.

A. Detection of PfHRP-2 in Clinical Samples

Histidine-rich protein 2 (HRP-2) is specific to P. falciparum (PfHRP-2) and is secreted into peripheral blood during parasite growth and development where it plays a role in heme detoxification (Rodriguez-del Valle et al., 1991). The antigen's widespread application in electrochemical and optical immunosensors is due to copious expression levels throughout the parasite life cycle. Although primarily abundant in blood, trace amounts can be found in cerebrospinal fluid, urine, and saliva of infected patients which offer an opportunity for non-invasive testing (Rodriguez-del Valle et al., 1991).

Nanoparticles are utilized in amperometric immunosensors (Sharma et al., 2008). Their small size and ease of immobilizing bioconjugate probes allow for increased surface concentration of enzyme-tagged detection antibodies, hence higher signals from the catalytic reaction of enzyme and substrate (Sharma et al., 2008). Sharma et al. were first to report an electrochemical immunosensor to detect PfHRP-2 in blood by amperometry (Sharma et al., 2008). The disposable immunosensor utilized multi-walled carbon nanotubes (MWCNTs) and gold nanoparticles (Nano-Au) to modify screen printed electrodes (SPE); resulting in Nano-Au/MWCNT/SPEs onto which rabbit-derived anti-PfHRP-2 were immobilized as capture antibodies (Sharma et al., 2008). A sandwich enzyme-linked immunosorbent assay format was employed for the biosensor with alkaline phosphatase (ALP)-conjugated antibodies. Amperometric measurements were applied using ALP hydrolysis of 1-naphthyl phosphate (Sharma et al., 2008). The Nano-Au/MWCNT/SPE had a limit of detection (LoD) of 8.0 ng/mL. This enhanced performance was attributable to the synergistic effect of MWCNTs and AuNP. More importantly, the immunosensor had a superior analytical performance compared with a commercial immunochromatographic lateral flow test in the analysis of microscopy positive patient sample (sensitivity: 96% vs. 79%, specificity; 94% vs. 81% respectively) (Sharma et al., 2008).

More recently, Hemben et al. used anti-PfHRP-2 monoclonal antibodies to capture PfHRP-2 at the surface of a screen printed gold electrode (Hemben et al., 2017). The captured antigen was targeted with HRP-labelled antibodies and the quantification of PfHRP-2 derived from the substrate (TMB-H2O2)-enzyme reaction by amperometry (Hemben et al., 2017). The LoDs in buffer and spiked human samples were determined as 2.14 ng/mL and 2.95 ng/mL, respectively (Hemben et al., 2017). Labelled antibodies were subsequently conjugated to gold nanoparticles (AuNP) to amplify the sensor signal which improved the sensitivity and LoD in buffer (36.0 pg/mL) and spiked serum samples (40.0 pg/mL) (Hemben et al., 2017).

B. Detection of pLDH in Clinical Samples

Plasmodium lactate dehydrogenase (pLDH) plays a catalytic role in the glycolytic pathway during the intraerythrocytic stages of Plasmodium (Palmer et al., 1998). It is produced by metabolically active parasites within infected red blood cells (RBCs) and has conserved catalytic residues in all Plasmodium spp. except in P. knowlesi (Palmer et al., 1998). pLDH is indicative of a recent infection and is generally cleared within 24 h of parasite clearance; hence, it more reliable in identifying recent unresolved infections (Palmer et al., 1998).

Aptamers are utilized in the detection of pLDH. Aptamers are smaller in size, thermostable, have extended shelf life without functional degradation, affordable, easily synthesized and can be readily modified. Hemben et al. functionalized screen-printed gold electrodes (SPGE) with anti-pLDH antibodies and applied a sandwich assay format to detect pLDH (Kanyong, 2020). The sensor initially achieved LoDs of 1.80 ng/mL in buffer and 0.70 ng/mL in serum. Application of colloidal AuNPs functionalized with HRP-labelled detection antibodies enhanced amperometric signals to LoDs down to 19 pg/mL (in buffer) and 23 pg/mL (in serum) (Kanyong, 2020).

C. Detection of GDH in Clinical Samples

Enzymes such as GDH in Plasmodium parasites play a role in in glutamate catabolism and ammonium assimilation (Britton et al., 1992). The enzyme is present throughout the sexual and asexual stages of the parasite development in significantly soluble quantities (Kanyong, 2020). Several structural and kinetic distinctions between host and parasite GDH makes it potentially useful in targeting live parasites (Kanyong, 2020).

A label-free capacitive aptasensor was constructed by graftting thiolated ssDNA aptamer (NG3) specific to *P. falciparum* (PfGDH) on a gold electrode (Kanyong, 2020). The sensor produced a LoD of 0.77 pM in serum with dynamic range 100 fM–100 nM (Kanyong, 2020). Subsequently, the process was integrated into an extended gate field effect transistor (EgFET). The NG3 aptamers were immobilized on an inter-digitated gold microelectrodes (IDμE) and connected to the FET to construct a sensitive and stable miniaturized aptaFET biosensor (Kanyong, 2020). A benefit of FET-type systems is that, it enables for sensitive and simple electrochemical measurements without requiring a typical redox marker (Kanyong, 2020). Following calibration curves in varying concentrations of PfGDH-spiked buffer and serum, a linear detection range of 100 fM–10 nM was obtained with LoDs in buffer and serum being 16.7 pM and 48.6 pM, respectively (Kanyong, 2020). The FET-based potentiometric sensor was highly selective in the presence of analogous human and plasmodial proteins, making it suitable for analysis of real sample for malaria diagnosis (Kanyong, 2020)

D. Detection of Aldolase

Aldolase plays key role in the glycolytic pathway of *Plasmodium* species where it catalyzes cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Kanyong, 2020). Targeting aldolase as an antigen in malaria has been largely confined to ICTs, however, an evaluation of four aldolase- and LDH-based commercial ICTs found variations in specificity to *P. vivax* ((Kanyong, 2020). A probable reason why aldolase biosensors have not received much interest could be due to the poor sensitivity reported in aldolase ICTs (Kanyong, 2020). The genes encoding aldolase in *P. falciparum* and *P. vivax* are highly conserved, making it a poor marker of differential diagnosis (Kanyong, 2020). This adds on to the growing recommendations of paralleled detection of malaria antigens in test devices in order to maximize sensitivity and specificity while reducing the risk of misdiagnosis (Kanyong, 2020).

E. Detection of Hemozoin in Clinical Samples

At the erythrocytic stage of its life cycle, the malaria parasites digest about 60–80% of erythrocytic hemoglobin resulting in the formation of heme and polymerized to insoluble hemozoin crystallites (Kanyong, 2020). Hemozoin is localized in parasite digestive vacuoles, therefore its presence in blood indicates a good marker of metabolically active *Plasmodium* parasites (Kanyong, 2020). The potential of surface-enhanced Raman spectroscopy (SERS) has been explored and shown to enhance the Raman signal of hemozoin by several folds (Kanyong, 2020). Exposure of parasitized RBCs to a gold-coated butterfly wing as SERS substrate produced Raman shift within malarial hemozoin pigment whereas uninfected lysates did not (Kanyong, 2020). The spectral markers of hemozoin from infected RBC

were detectable at the early-ring stage parasitemia levels of between 0.0005% and 0.005%. While enhancements of Raman signals occur when hemozoin crystals are in direct contact with metal surfaces, another SERS method that applied synthesized silver nanoparticles inside parasites to achieve a close contact with hemozoin demonstrated an ultrasensitive hemozoin detection at 0.00005% parasitemia level in the ring stage (2.5 parasites/μL) (Kanyong, 2020). These SERS methods have shown potential in early malaria diagnosis at low parasitemia levels, however, Raman spectrometers, and particularly those with high spectral resolutions, are expensive (Kanyong, 2020). The paramagnetic properties of hemozoin crystals have been exploited for label-free detection using magnetic resonance relaxometry (MRR). In combination with a microfluidic setup, the MRR system achieved an accurate early detection at a parasitemia level of 0.0005%((Kanyong, 2020).

III. CONCLUSION

The steady rise in malaria cases each year across the world makes rapid and accurate testing of malarial infections a paramount matter. Although RDTs have been shown to be effective rapid detectors of malaria parasites, they do not offer the versatility that is emerging with biosensors and immunosensors. This is because the latter two not only rapidly detects malaria parasites but also; due to their high precision in targeting specific parasitic elements, they can differentiate what growth stage the parasite is, chronicity of infection and whether radical cure is achieved. Further, they achieve this independent of parasitic load and concomitantly also gives a picture of the parasitemia. Out of the several parasitic elements, malaria biosensors have mostly been made to target pfHRP-2, pLDH, GDH, hemozoin and aldose enzyme. pfHRP-2 biosensors offer superior analytical performance but are limited by the fact that the protein has a longer duration in circulation even if parasites are cleared and that there have been reports of mutation. pLDH biosensors are equally effective especially in detecting recent infections since the target enzyme has a 24hr period before it is cleared. GDH biosensors may be effective in detecting live parasites whichever their life cycle stage. Aldose based biosensors are however not commonly used due to poor sensitivity that has been reported. Hemozoin biosensors are also effective especially in detecting metabolically active parasites in circulation. Overall, these results from these biosensors and immunosensors guide the choice of treatment to be used which reduces emergence of resistance and optimizes radical cure.

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