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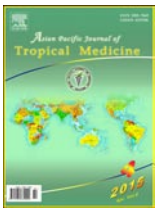

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A brief review on biomarkers and proteomic approach for malaria research

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ABSTRACT

Malaria remains as one of the significant health threat to people living in countries throughout tropical and subtropical zones. Proteomic studies of *Plasmodium*, the protozoan causing malaria, is essential for understanding its cellular structure, growth stage-specific expression of protein metabolites and complex interaction with host. In-depth knowledge of the pathogen is required for identification of novel biomarkers that can be utilized to develop diagnostic tests and therapeutic antimalarial drugs. The alarming rise in drug-resistant strains of *Plasmodium* has created an urgent need to identify new targets for drug development that can act by obstructing life cycle of this parasite. In the present review, we briefly discuss on role of various biomarkers including *Plasmodium*-associated aldolase, histidine-rich proteins and lactate dehydrogenase for diagnosis of malaria. Here we also summarize the present and future prospects of currently used techniques in proteomic approaches such as two dimensional gel electrophoresis and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) for diagnosis and potential identification of novel proteins for malaria research.

1. Introduction

Malaria is an ancient vector-borne disease of humans and other primates caused by a highly complex protozoan belonging to the genus *Plasmodium*[1,2]. Female *Anopheles* mosquitoes are insect vector for transmitting the disease in humans. The disease is endemic to more than 100 countries throughout tropical and subtropical zones. Recently, the world malaria report released by world health organization has estimated that around 3.4 billion people are at risk of malaria and about 207 million cases of malaria occurred globally during 2013[3]. In Africa significant numbers of child death has been directly attributed to malaria. A research suggests that majority of malaria-associated deaths globally were children less than 5 years of age from sub-Saharan Africa[4]. Nonetheless, even after several decades of the disease control campaigns, malaria persists as one of the most serious public health problem not only in endemic countries but also in non-endemic regions where the increasing number of imported malaria cases via tourists and immigrants are on the rise[5].

Four species of the pathogen namely: *Plasmodium vivax* (*P. vivax*), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium malariae*, and *Plasmodium ovale* (*P. ovale*) are highly distributed among malaria-affected regions in the world. In addition, recently discovered *Plasmodium knowlesi*-associated zoonotic form of human malaria in Southeast Asia is also creating serious concerns among pathologist and epidemiologists[6]. Among the species, *P. falciparum* accounts almost all of the malaria-associated mortality[7,8]. Malarial pathogen has exceptional ability to adopt and maintain a complex parasite life cycle in both humans and *Anopheles* spp. with several morphologically and functionally distinct extra- and intra-cellular stages. In addition, mutational selection resulting into drug resistant strains of the parasite, the spread of insecticide resistant mosquitoes and lack of effective vaccines against the *Plasmodium* spp. are some of the critical barriers preventing eradication of this disease[9].

Morbidity and mortality caused by the disease is exclusively

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associated with the erythrocytic stage of infection where the pathogen continuously undergoes intracellular development and re-invasion into red blood cells (RBCs) resulting in exponential parasite proliferation. If untreated, the disease progressively causes severe haemopenia, kidney failure, mental confusion,seizures, coma, and death[10,11]. Multiple neurological complications and cognitive deficits can arise specially during cerebral malaria as the parasite can also infect brain. In addition, the parasite is known to constantly develop resistance against antimalarial drugs making it more difficult to cure the disease[12]. Hence, it is clear that early diagnosis and prophylaxis has a major role in preventing malaria-associated mortality. Even though several rapid detection tests (RDTs) and kits are available for screening malaria infection, their efficiency and accuracy are still questionable. Thus, there is a growing need for exploring new detection techniques that are highly sensitive and portable for early identification of malaria. In this context, an in-depth study of potential biomarkers associated with malaria pathogen can be crucial for identification of a suitable biorecognition element and its implementation for devising a robust and reliable diagnosis technique for early detection of the disease. Here, we briefly discuss on the current prospect and future potential of biomarkers and different proteomic approaches that can be used for malaria research.

2. Drugs used for treatment of malaria

Parasite causing malaria exhibits astonishing adoption abilities and due to prevalence of several species, strains, and variants the treatment of malaria is not always easy. Even today, the choice of drugs for effective medication is quite limited and drug resistant strains of this pathogen are on the rise[12,13]. The drug of choice Aralen (*Chloroquine phosphate*) is effective for all malarial parasites except for chloroquine-resistant *Plasmodium* strains[9]. Traditional medication for *P. falciparum*-mediated malaria involved the use of sulphadoxine-pyrimethamine (SP) or chloroquine. However, the over-dependence and excess use of both quinoline compounds (amodiaquine, chloroquine, mefloquine, and quinine) and antifolatedrugs (chlorproguanil, sulphonamides, and pyrimethamine) have led to cross-resistance among these compounds[14,15]. Emergence of such resistant strains of *P. falciparum* has motivated to switch towards new class of artemisinin-derived antimalarial compounds. Currently, the artemisinin-derived combination therapies (ACTs) which involves use of artemisinin derivatives in combination with other traditional antimalarial drugs, is regarded as the best treatment option[16]. For *P. vivax* and *P. ovale* which are known to possess dormant stages and can cause relapses, primaquine is often administered in addition to ACTs. Unfortunately, any therapy is not always guaranteed to work against the disease since several strains of the parasite resistant even to ACTs have been reported in different parts of the world[16,17]. Although several strategies and multiple drug treatment protocols do exist to prevent drug resistant malaria, detecting the disease at its early stage remains vital for the successful treatment. Hence, in this scenario the importance of biomarker-based technique for timely diagnosis of the disease becomes more essential.

3. Role of biomarkers in malaria diagnosis

Biomarkers can be defined as any measurable changes for molecular, biochemical or cellular alternations in biological samples that can indicate biological, pathological or therapeutic responses[18]. Possibility to detect certain biomarkers for *Plasmodium*-mediated infection at early stages of malaria can be crucial for formulating disease management strategies and choosing correct prophylaxis for the disease[19]. Typically for the endemic zones of Africa where asymptomatic malaria is rampant, such biomarker-based detection techniques combined with proper treatment strategies may significantly help deplete human reservoirs of the parasite that frequently contributing to persistence of malaria transmission in such areas[19-21]. Current advancement in proteomics and immunotechnology has provided vital tools for in-depth study and analysis of different human body fluids including saliva, urine, and serum. In general, analysis of proteome from such fluids provides a valuable resource for the identification of potential disease-related markers. The host immune response against the disease condition can exhibit rapid alteration in expression pattern unique for a pathogen which may be directly correlated with disease progression at its early state[22]. The use of biomarkers provides us with sensitive and effective means for investigation of disease pathogenesis. Currently, several malaria-associated biomarkers have been utilized for the disease identification and few of them are briefly described below.

3.1. P. falciparum lactate dehydrogenase (PfLDH)

Plasmodium is a voracious scavenger of blood glucose that can force the RBC to increase glucose consumption up to 100-fold during intraerythrocytic cycle. Microarray experiments using *P. falciperum* transcriptome has shown that all enzymes in glycolytic pathway are significantly upregulated during early trophozoite stage correlating with the high metabolic state of the pathogen at this particular stage of its asexual life-cycle[23]. In particular, the enzymes involved in energy metabolism during the intraerythrocytic stages of the parasite lack a functional citric acid cycle, and production of ATP depends fully on the glycolytic pathway[24]. Among other enzymes, the pathogen seems to principally rely on *Pf*LDH as its essential enzyme in glycolytic pathway to convert pyruvate into lactate[25]. The *Pf*LDH RNA expression level gradually increases and reaches its peak within 24 to 30 hours of infection during the intra-erythrocytic cycle. This expression subsequently declines tozero in the schizont stage[20,26]. Since the *Plasmodium* LDH (*p*LHD)

contains some major structural differences compared to human form of enzyme, this biomarker holds therapeutic potential to formulate promising antimalarial drug targets or diagnostic tools[27,28]. The malarial LDHs of parasite has been discovered to contain five-residue aminoacid insert known as D-aspartic acid, E-glutamic acid, K-lysin, W-tryptophan, and N-asparagine (DKEWN) on one of its active site and the insert has also been employed as a common diagnostic epitope for *Pf*LHD[29,30]. Contrary to the human LHDs, investigation of enzyme kinetics has revealed that the *p*LDH enzyme is not significantly affected by the excess substrate pyruvate[31]. This feature of reduced substrate inhibition is attributed to a single amino acid substitution in the *Plasmodium* enzyme (Ser163Leu). The *p*LDH is particularly distinguished from mammalian LHD due to presence of long substrate specificity loop[31,32]. It is clear that *p*LHD with its unique structural features, metabolic differences, and enzyme kinetics presents as an attractive biomarker for antimalarial drug design.

3.2. Histidine-rich proteins (HRPs)

The HRPs relating to *Plasmodium* were first discovered during the analysis of cytoplasmic granules from the avian malaria parasite *Plasmodium lophurae*. The polypeptides in granules contained abnormally high levels of histidine reaching upto 73% in total[33]. There are three major types of HRPs associated with *Plasmodium* parasite and named according to the order of their discovery[24,34]. The HRP1 is basically aKnob-associated protein present in Knob-positive strains of the parasite and is known to assist coadhereance of infected erythrocyte to venular endothelial cells[20,35]. The HRP2 is regarded as a vital biomarker unique to *P. falciparium* and is present in both Knob-positive and -negative strains of the parasite that causes the most severe form of malaria[36]. The *P. falciparum* HRP2 (*Pf*HRP2) is known to exhibit many functions including tightly binding with glycosaminoglycans (heparan sulfate,dermatan sulfate, and heparin) causing inhibition of antithrombin and detoxification of heme by forming haemozoin[2]. HRP2 has remained one of the prime targets for development of antimalarial drugs and rapid diagnostictests (RDTs) as evidence shows that *Pf*HRP2-based assays are more sensitive towards detection of *P. falciparum* than aldolase- and LHD-based diagnostic tests[37]. However, the *P. falciparum* from Asia-Pacific region is known to express high variability in *Pf*HRP2, which has posed significant difficulty for the development of efficient malaria RDTs development[38]. Lastly, the HRP3 is not as common as HRP1 and HRP2. *P. falciparum* HRP3 (*Pf*HRP3) is a small histidine-rich protein (SHARP) that shares several structural homology with PfHRP2. It has been suggested that both *Pf*HRP2 and *Pf*HRP3 were derived from duplication and interchromosomal divergence from a common ancestral gene[38-40]. Due to structural homology, several antibodies against *Pf*HRP2 are known to cross-react with *Pf*HRP3, and thus contributing to the detection of *P. falciparum*[41,42]. Overall, the *Plasmodium*-associated HRPs constitute a prominent biomarker for malaria diagnosis.

3.3. Haemozoin

Haemozoin is often known as malaria pigment as it is a visible marker in detection of malarial parasite. It is a non-toxic insoluble microcrystalline by-product formed by polymerizing free-toxic heme after digestion of haemoglobulin by *Plasmodium* and some other RBC-feeding parasites[10,43,44]. Several currently available antimalarial drugs including mefloquine and chloroquine act by blocking the haemozoin biocrystallization that kills malaria parasite[45]. Even though the exact process for formation of haemozoin is barely understood, the HRP2 is suggested to assist initiation of haemozoin formation by firmly binding to heme molecules and initiating their polymerization. However other haemozoin synthesis mechanism based on neutral lipid bodies and polar membrane lipids have also been reported[46-48]. As the haemozoin formation is crucial for the parasite survival, it has therefore become a prominent target for antimalarial drug development.

3.4. Plasmodium aldolase

Proteins and enzymes associated with the glycolytic pathways of the *Plasmodium* has remained a prime focus for parasite detection and antimalarial drug development. Aldolase has a vital role in cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in the glycolytic pathway of the parasite. The *Plasmodium* aldolase is a homotetrameric in nature with each subunit of approximately 40 kDa[49]. Unlike vertebrates which comprise three tissue-specific aldolase enzymes, the *P. vivax* and *P. falciparum* contain only one type of aldolase isozyme. The enzyme is 369 amino acids in length and their nucleotide and amino acid sequences are relatively conserved[50,51]. The enzyme can be found either as an insoluble membrane-bound aldolase or localized in cytoplasm of the parasite as an active and soluble form[49]. The plasticity of active-site region and multimeric nature of the enzyme is also suggested for its intriguing non-enzymatic activity for assisting the invasion machinery of the malaria parasite[52]. The *Plasmodium* aldolase exhibits drastic difference from the host enzyme and thus has been utilized in several commercially available RDTs. Even though several reports have suggested that aldolase-based diagnostic tests provide relatively lower sensitivity as compared to HRPs-based tests, the enzyme remains a vital target for disease diagnosis and drug development[53]. Thus, the *Plasmodium* aldolase has been regarded as prominent biomarker in malaria research.

3.5. Glutamate dehydrogenase (GDHs)

The GDHs are ubiquitous enzymes responsible for the reversible

oxidative deamination of L-glutamate for production of α -ketoglutarate and ammonia utilizing NAD (H) or NADP (H) as co-factors[54]. The enzyme serves as a branching point of nitrogen-carbon metabolism as it can either assimilate ammonia and provides glutamate for storage of nitrogen or dissimilates ammonia and generates α -ketoglutarate as a feed for tricarboxylic acid metabolism. Since malaria parasite exhibits different isoforms of GDHs, these enzymes are considered as an important biomarker for malaria diagnosis.Both NADP- and NAD-dependent GHDs are present in *Plasmodium*. In particular, the *P. falciparum* exhibits three types of isozymes encoding GDHs. Two genes *gdha* and *gdhb* encoding potential GDHs are located in chromosome 14 and the third gene *gdhc* is present in chromosome 8[7,54]. The *Plasmodium* GDHs contains *N*-terminal residue unique to the parasite that is present throughout intra-erythrocytic cycle of the pathogen. As RBCs do not contain GDHs, it can potentially serve as a biomarker for disease diagnosis[55,56]. Techniques involving western blotting, immunochromatographic assays have been utilized to detect the parasite utilizing GDHs[8,57]. Hence, the GDHs may be considered as a significantly important disease-associated biomarker for malaria diagnosis and treatment.

4. Role of proteomics in malaria research

Proteome generally refers to the entire set of proteins belonging to a cell or an organism which can vary with time depending on distinct requirements or stresses that it endures[58]. Proteomics provides necessary tools for large-scale experimental analysis of the proteome for generating data on relevant protein sets from pathogen and the host[59]. This information can help understand the host-parasite relation in context to the structures, specific expressions, and functions of the proteins. The past decade has witnessed astonishing increase in study and global shearing of knowledge on *Plasmodium* proteome leading to establishment of global protein database of malaria parasite[60]. This has significantly helped researchers by assisting identification of potential targets for antimalarial drugs that specifically interrupt the host-parasite interaction and thus prevent disease development[61]. Reports have suggested that the survival of malaria parasite requires tight regulation of several proteins during different stages of its life-cycle in both vertebrate and invertebrate host[1,61]. However, most of today's modern technologies in proteomics for detection and prevention of malaria are focused on intra-erythrocytic stage of the parasite life-cycle which is the major cause of malaria-associated mortalities[59]. Thus, the techniques that can be applicable both for research and clinical diagnosis of malaria are of immense importance for combating this disease.Some of the major modern proteomics-based approaches for research on pathogenesis and diagnosis of *Plasmodium* infection are summarized below.

4.1. One dimension sodium dodecyl sulphate-polyacrylamide gel electrophoresis (1D SDS-PAGE) approach in malaria research

Proteomic approach requires identification and ascertaining the protein population that are differentially expressed and also detection of any post-translational modifications that occur during each stage of parasites life-cycle[11,62]. The1D SDS-PAGE is a widely used technique in molecular biology to separate biological macromolecules, usually proteins or nucleic acids (Figure 1). The technique has been frequently employed for separating and analyzing various protein fractions from trophozoite stage of *P. flaciparum*[11]. In addition, it has also been used for identification of phosphorylated protein pattern by separation of phosphoproteome of *P. falciparum* parasitized RBCs[63]. The method has application in protein separation for detection of distinct patterns of blood-stage *Plasmodium* antigens using plasma immunoglobulin-G (IgG) subclasses from individuals with different level of exposure to *P. falciparum* infections[64]. The 1D-SDS PAGE has remained a well-adopted method for separating the protein fractions derived from *Plasmodium*-infected samples to enhance sensitivity and resolution of protein identification prior to mass spectrometry[63,65]. Taken together, the 1D SDS-PAGE presents itself as a simple yet highly applicable technique for study of malaria proteomics.

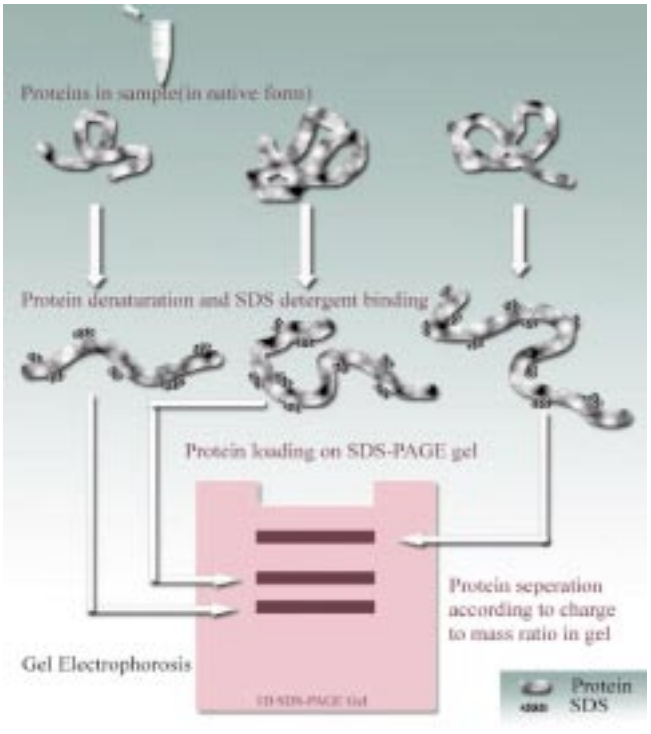

Figure 1. Schematics of one dimensional SDS-PAGE.

The sample containing protein mixtureis treated with ionic detergent sodium dodecyl sulfate (SDS) which denatures and binds to proteins in a fixed charge to mass ratio making them evenly negatively charged. The samples are then added to wells at the top of the polyacrylamide gel. The electric field is then applied which causes different proteins to separate into bands as they move down through the gel matrix. These bands of separated proteins can then be stained and compared or even used for other detection steps.

4.2. Two dimensional SDS-PAGE (2D SDS-PAGE) approach in malaria research

The 2D SDS PAGE is a widely used two-dimensional gel electrophoresis (2-DE) technique for investigating *P. vivax* and *P. falciparum* infected blood samples[66,67]. It is also one of the most commonly used methods for obtaining a snapshot of proteome at the specific time of infection. A basic schematic of the assay is given in Figure 2. Customized forms of 2D SDS-PAGE has been utilized as a semiquantative gel electrophoresis technique for assisting proteomic profiling of *P. falciparum*[68]. In addition, the mass spectrometry-based techniques such as matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) preferentially require the samples to be pre-processed using 2D SDS-PAGE for analyzing the bimolecular content in high resolution[63,69]. Moreover, the first pioneering study on protein maps from proteomes of merozoites and schizonts of *P. falciparum* were obtained utilizing 2D-PAGE and mass spectrometry[70,71]. Recently, the 2D SDS-PAGE approach along with immunoflurosence assay has been utilized to identify and investigate the expression and localization of a relatively conserved heat shock protein in malaria parasite[72]. Likely, in a separate report, the 2-DE has been implemented as a method of choice for proteomic approach to study malaria by investigating protein expression pattern in *P. falciparium* under treatment of antiplasmodial drug such as quinine and mefloquine[73]. Thus, is it clear that 2-DE serves as an essential tool for malaria research with proteomic approach.

4.3. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The LC-MS/MS is basically a hybrid analytical technique that contains physical separation capabilities of high performance liquid chromatography (HPLC) to fractionate the samples into individual analytes while retaining mass analysis capabilities of tandem mass spectrometry to quantitate these analytes[74]. A generalized schematic of the technique is given in Figure 3. The quest for rapid screening of multi-biomarker profiles for various diseases diagnosis has led to increasing use of LC-MS/MS for both clinical and research purpose[75]. The technology enables researcher to undertake advance proteomic approaches by investigating large-scale protein expression profiles with high accuracy for complex organism like *Plasmodium*. The LC-MS/MS in combination with 1D-SDS PAGE has previously been used for proteomic analysis of salivary gland proteins extracted from urban malaria vector *Anopheles stephensi*[76]. Similarly, the technique has also been employed for investigation of the *P. vivax* schizont stage proteome in humans for identification of immunogenic proteins that may hold therapeutic potentials[62]. Their computational research data provided first direct insight into identification and functional characterization of salivary proteins[76]. LC-MS/MS analysis of *P. falciparum* extracellular secretory proteins produced during asexual blood stages of parasite has assisted in revealing numerous proteins potentially involved in host immune modulation and signaling[77]. In addition, the technique has also been utilized for study of plasma and cerebrospinal proteomes from children acquiring cerebral malaria for better understanding of pathogenesis and helps develop more-specific diagnostic methods[78]. Hence, the technique can be regarded as a rapid yet highly efficient tool for large-scale proteomic analysis for malaria.

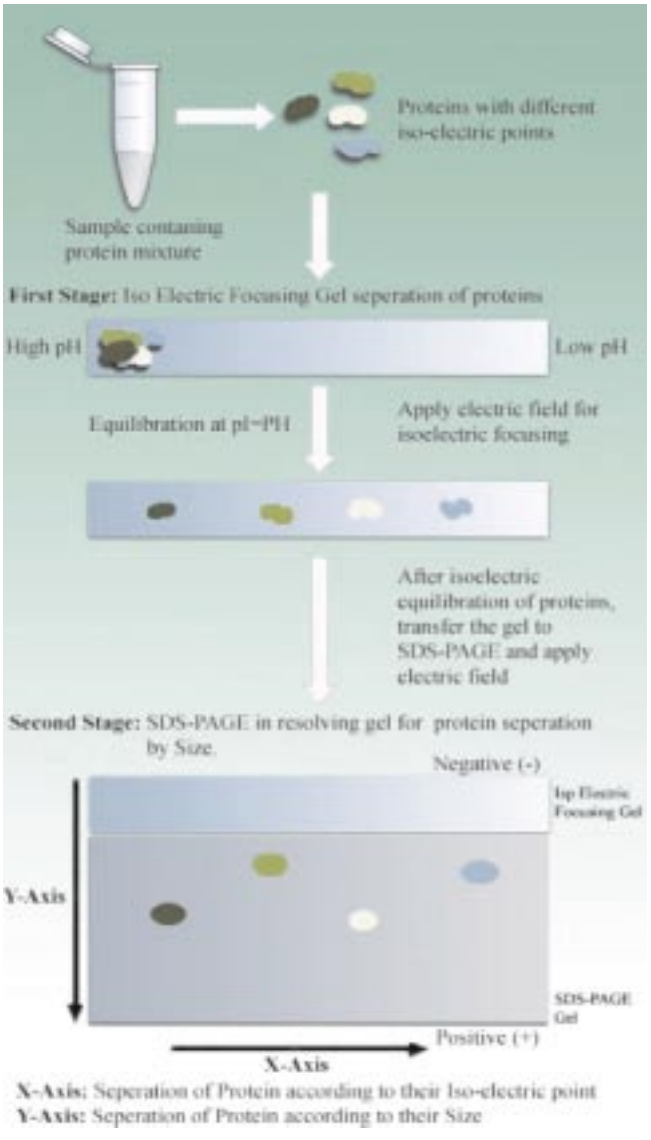

Figure 2. Schematics of two dimensional SDS-PAGE.
The process contains two major steps. At first the sample is loaded on a thin gel having pH gradient and electric current is applied which separates the proteins according to their isoelectric point (pI). At this point, the pI of a given protein is in equilibrium with pH of the gel. In the next step, the gel strip is then loaded onto another polyacrylamide gel and second electric current is applied in a direction perpendicular to the first. In this case, the proteins previously separated according to their pI migrates from initial gel into the second gel and resolve depending on their masses. The separated proteins appear as a spot which can either be stained and visualized or used for further detection steps.

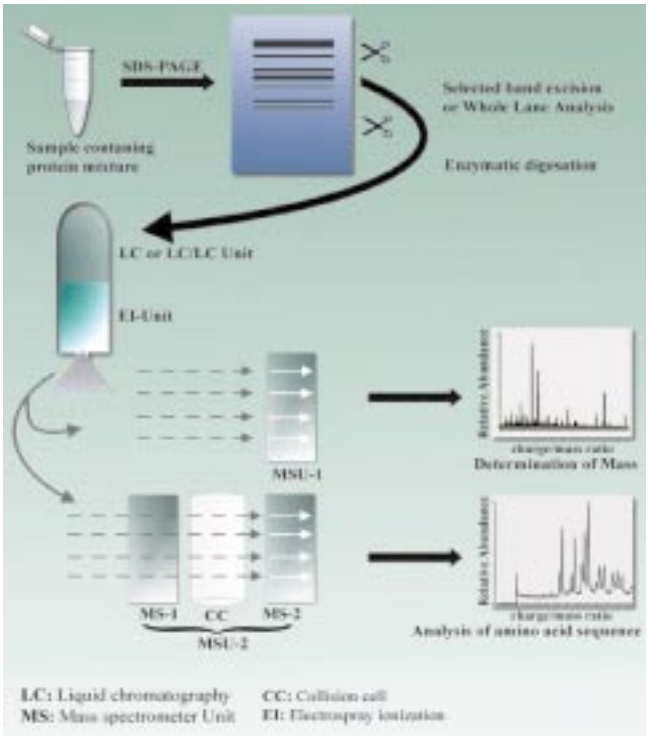

Figure 3. Schematics of LC-MS/MS.

The samples are initially separated using SDS-PAGE to obtain bands of protein followed by enzymatic digestion. After digestion, the resultant product of individual band or whole lane is subjected to multi-dimensional liquid chromatography (LC/LC) for further separation of the mixture. The elute obtained from LC/LC unit is further ionized using EI-Unit. Each elute peak can be then analyzed for its mass using MSU-1. Likely, for determination of peptide unit, the elute peak is subjected to MSU-2 where it is first separated in MS-1 and passes through collision chamber where it is further fragmented and subsequently analyzed by MS-2. Finally, the data from MS-2 is computed to obtain peptide sequence.

4.4. MALDI–TOF

The technique is based on ionization of biomolecules, generally proteins, in samples, followed by analysis of its constituent using specially designed mass spectrometric equipment that utilizes high energy collision-induced dissociation to investigate amino acid sequence of peptides[79]. Figure 4 represents generalized schematics of MALDI-TOF mass spectrometry (MS). Due to its accuracy, speed and cost effectiveness, the technique has been successfully employed as an identification procedure in clinical microbiology and also widely implemented in routine laboratory practices[79,80]. The technique along with SDS-PAGE has been applied for characterization of the *P. vivax* erythrocytic stage proteome and identification of a relatively stable protein PV180L as a potent immunogenic antigen during the erythrocytic cycle of malaria parasite[81]. Previously, it was reported that the method had been employed to assist analysis of recombinant *P. vivax* merozoite surface antigen protein that was allowed to be expressed in *Escherichia coli* for malaria vaccine development research[82]. Recently, MALDI-TOF has been employed for rapid identification of *Anopheles* mosquitoes from its leg protein samples and has been able to generate several sets of biomarkers for its precise identification[83]. The *P. falicaprum* and *P. vivax* proteomes subjected to MALDI-TOF analysis have revealed several proteins that are differentially expressed and are potentially important for studying disease pathogenesis[66]. In a separate report, the technology has been applied to assist identification and extend the knowledge on redox proteins such as pthioredoxin, glutaredoxin and plasm oredoxin for better understanding of redox interactome in malarial parasites[84]. Thus, reports presented here clearly demonstrate that MALDI-TOF is a relevant tool for studying malaria proteomics and opens new avenues for both clinical diagnosis and experimental anti-malarial drug development.

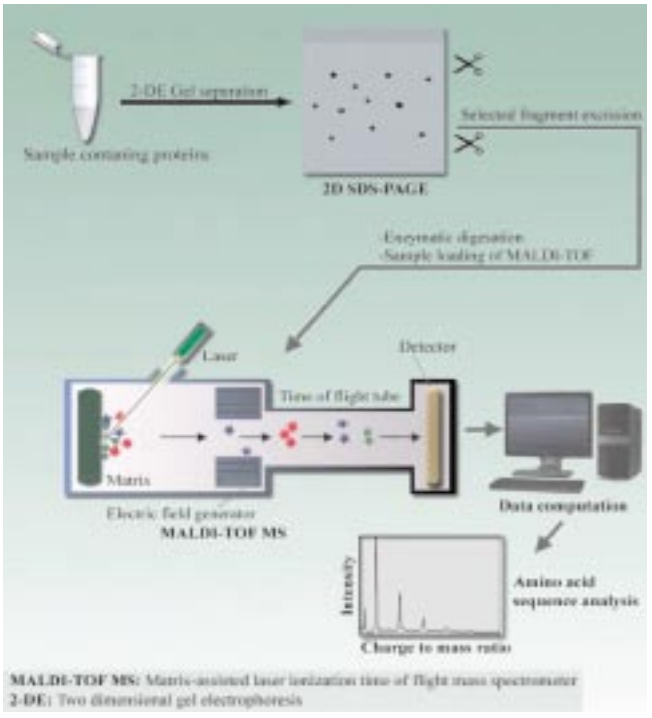

Figure 4. Schematics of MALDI-TOF.

Samples are initially processed using SDS-PAGE to obtain bands of protein which is then enzymatically digested. The resultant product of individual band or whole lane is subjected to MALDI-TOF MS where the proteins placed on matrix molecule in solution are allowed to undergo soft ionization using laser. The ions from protein accelerate under the influence of electric field as they pass on through the time-of-flight tube where the charge-to-mass ratio of each ion is determined using time measurement by the detector. Finally, the data is computed to determine the mass and polypeptide sequence of the protein.

4.5. Surface-enhanced laser desorption/ionization time of flight (SELDI–TOF)

The SELDI-TOF MS is a modified form of MALDI-TOF that

utilizes target surface modified to achieve biochemical affinity and selectively analyze samples based on their affinity towards the surface matrix[85,86]. Generalized schematic of the technique is given in Figure 5. One of the obvious advantages of this surface-enhanced process is that components, such as salts or detergents that commonly cause problems with other analytical tools, are removed prior to analysis[87]. The technology is characterized by its rapid diagnostic capabilities that can be applied to study proteomes to discover biomarkers associated with parasitic diseases which may include host proteins or protein fragments derived from parasite or host, in body fluids or tissues following infection[88]. In comparison with other proteomic techniques, SELDI-TOF has several key advantages including ability to analyze complex biological samples with minimal pre-processing, ease of handling, and high throughput[86]. The method had been used for protein expression profiling to identify chloroquine resistance markers in *P. falciparum* strains resistant to chloroquine[9]. Recently, the SELDI-TOF has been used to identify hepcidin, a hepatocyte-associated protein having a function of sequestration of iron, from blood plasma for its possible role in anti-inflammatory response in childhood malaria[89]. The technique has been employed for determining urinary hepcidin in malaria patient suffering from uncomplicated *P. falciparum* infection to study the relationship between parasitemia and anemia[90]. Moreover, the level of sophistication behind this technology has assisted researchers in proteomics to explore previously uncharted conditions in patients chronically infected with blood-borne protozoan parasites including malaria[88,91]. Thus, it is clearly evident that the protein expression profiling approach using SELDI-TOF provides a useful tool for understanding the proteome of malarial parasites.

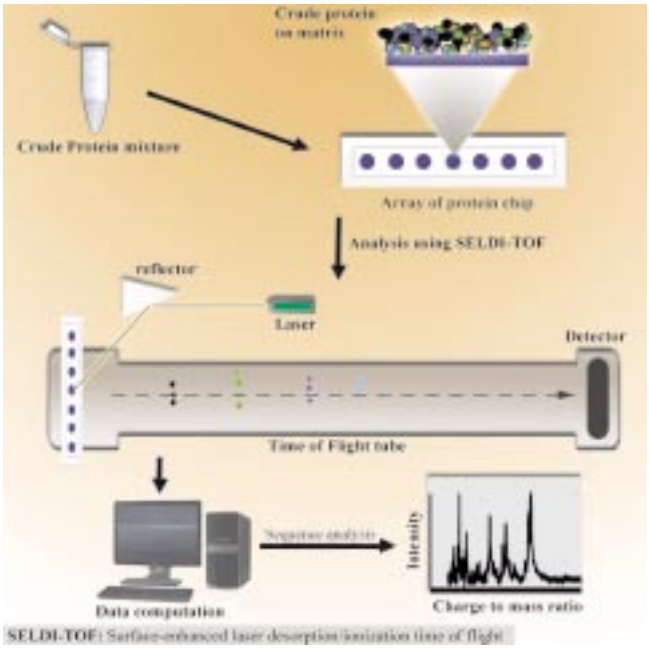

Figure 5. Schematics of SELDI-TOF.
Samples are initially allowed to attach and crystallize on specifically designed surface matrix of a protein chip for enhancing biochemical affinity of specific proteins or analytes from a complex mixture. Array of such protein chips are lined into the SELDI-TOF mass spectrometer where the protein on matrix is allowed to undergo soft ionization using laser. Once ionized, the protein particles accelerate under the influence of electric field as they pass on through the tube and charge-to-mass ratio of each ion is determined using time-of-flight mass spectrometry by the detector. The resulting data is computed to determine the mass and polypeptide sequence of the protein.

5. Conclusion

The advancement in techniques for investigating proteome of *Plasmodium* and identification of potential biomarkers has undoubtedly widened our knowledge on malaria research. Protein profiling and characterization of stage-specific proteome of the parasite has helped researchers to reveal numerous novel molecules that may hold significant potential for targeted therapeutic drug development.The level of sophistication and sensitivity offered by current technologies for proteomic studies can help researcher to deeply explore the malaria pathogenesis and possibly assist identification of new targets and strategies for devising reliable diagnostic tests. Overall, the review provides a brief overview on the current scenario and future prospects of malaria biomarkers and various proteomic approaches used for malaria research.

Conflict of interest statement

We declare that we have no conflict of interest.

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