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Chapter

Plasmodium falciparum: Experimental and Theoretical Approaches in Last 20 Years

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Abstract

Malaria, the severe vector-borne disease has embedded serious consequences on mankind since ages, causing deterioration of health, leading to deaths. The causative parasite has a wide distribution aligned from tropical to subtropical regions. Out of all the five species *Plasmodium vivax* and *Plasmodium falciparum* have registered about more than 600 million cases worldwide. Throughout the decades, identification of various antimalarial drugs, targets, preventive measures and advancement of vaccines were achieved. The key to executing malaria elimination is the appropriate laboratory diagnosis. Development includes positive scientific judgments for a vaccine, advanced progress of 3 non-pyrethroid insecticides, novel genetic technologies, possibilities to alter malaria parasite mediation by the mosquito, identification of drug resistance markers, initiation of *Plasmodium vivax* liver stage assessment, perspective to mathematical modeling and screening for active ingredients for drugs and insecticides. Although the last century witnessed many successful programs with scientific progress, however, this was matched with notable obstacles. The mutation in the genes has changed the overall gameplay of eradication. This chapter aims to examine the numerous experimental and theoretical works that have been established in the last two decades along with the ongoing methodologies consisting of detailed explanations necessary for the establishment of new targets and drugs.

Keywords: *Plasmodium falciparum*, vector-borne, protozoa, vaccine

1. Introduction

Malaria has been a curse to mankind since early ages. It is initiated by a protozoan parasite, infecting the human circulation system. The disease is distributed throughout the tropical and subtropical regions, with transmission caused mainly through the *Anopheles* mosquitoes [1, 2]. In 2018, there were cases of about 228 million around the world which resulted in deaths of about 405,000 people. Initially four different parasite species were reported for transmission. Recently, *Plasmodium knowlesi*, a simian parasite, has been identified as a sporadic source of certain human infections, in the areas of Southeast Asia [3, 4], thus, raising the number to five species. The

Plasmodium falciparum species are spread worldwide and has an ability to inhibit the host physiology during the infection, making it more fatal as compared to other species [5].

Experimental study in 1991 showed that *Plasmodium falciparum* gradually evolved from two avian parasites which later got transferred to human [6]. However, in 2011, it was found that the parasite *P. falciparum* actually arose from *P. praefalciparum* rather than directly originating from the avian parasite and eventually got segregated into human parasites. The parasite basically infects humans, gorillas and chimpanzees, and *P. falciparum* of the *Laverania* family is the only species that flourishingly got transferred from apes to humans [7].

Development of vaccines is another way to overcome the burden of malaria in lieu of drugs. Unfortunately, the complicated biology, diverse genome and the life cycle of the malarial parasite has been obstructing the vaccine development process thus; no effective vaccination (licensed) has been approved against malaria [8]. The available vaccines can be categorized into: attenuated forms of microbes, inactivated microbes or protein-based subunit [9]. Recent parasitologist had made an attempt to manufacture a vaccine that can produce entire parasites in adequate quantities to stimulate immunity [10].

1.1 Treatment regimes

A remarkable progress has been achieved in the mission of eradication of malaria from mosquito nets to artemisinin-based combination therapies (ACTs). In 1820, Quinine, the world's first anti-malarial drug was introduced. It was isolated from the cinchona's bark and was administered as tonic water. Later on, various natural as well as synthetic compounds were introduced [11]. However, the development of drug resistance for the existing drug led to a failure of the treatment. Drugs with improved efficacy and potency were introduced to deal with the resistance. Recent study on drug resistance showed that *de novo* resistances are appearing more rapidly [12, 13]. The first case of drug resistance ever reported was in 1980, it was against Quinine [14].

In order to overcome drug resistance, the antimalarial drugs were administered through a combination treatment called Artemisinin-based combination therapy (ACT). The artemisinin and its derivatives compounds are still used as antimalarial drugs during the parasite stages [15, 16]. The world health organization (WHO) still suggests the use of ACT against *falciparum* [17]. Currently, *Plasmodium falciparum* has almost developed resistance for every drug available [18]. The list of drug molecules undergoing clinical trials (last two decades) is given in **Table 1** [19]. So, there is a desperate need for new antimalarial drugs in the market.

To tackle the high morbidity and mortality caused by malarial parasites chiefly in young children, a global malaria control strategy, prompt and explicit diagnosis with treatment of malaria is indispensable to accomplish a convenient cure, along with developing numerous drugs against it. However, various surveys described and reported resistance for pretty much all available antimalarial drugs that broaden the vital requirement for the development of new antimalarial drugs against substantiated existing targets, and to initiate search for novel targets in order to eradicate the speedily mutating parasites.

In this chapter, we have discussed the recent computational as well as experimental procedures that have taken place in order to facilitate the diagnosis along with the development of new antimalarial drugs in the last two decades.

Drug Name	Year	Mechanism of Action
AQ 13	2007	Not resolved so far
OZ277+ Piperaquine	2007	check the *Pf*-encoded sarcoplasmic endoplasmic reticulum calcium ATPase
MMV048	2012	parasite enzyme phosphoinositol 4-kinase enzyme inhibition
Albitiazolium (SAR9727)	2012	Restricting choline transfer within the parasite
P218	2012	*Pf*DHFR inhibitor
KAF156 Ganaplacide/ lumefantrine	2014	Not yet resolved
SJ733	2014	The P-type Na + –ATPase transporter
Sevuparin (DF02)	2014	Anti-adhesive polysaccharide derived Blocks merozoite invasion and sequestration
M5717 (DDD498)	2015	It inhibits PfeEF2, where an attaching pocket for M5717 was not explained.
KAE609 (cipargamin)	2015	Na + -TPase 4 ion channel-inhibitor
Fosmidomycin + Piperaquine	2015	DOXP pathway
Artefenomel (oz439) + Piperaquine	2016	Synthetic endoperoxide
DSM265	2017	Suppress dihydroorotate dehydrogenase enzyme
Methylene Blue	2017	Inhibits *P. falciparum* glutathione reductase to check haem polymerization
MMV390048	2017	Phosphatidylinositol 4-kinase (PfPI4K) inhibitor

Table 1.
List of the ongoing clinical trial carried out for as drug against malaria in the last two decades (2000–2020).

2. Experimental approaches concerning detection of malaria

For the detection and diagnosis of malaria-parasite infections and to tackle it effectively, multiple experimental procedures have been established to meet the specific demands during the entire diagnosis process. In this chapter we have mentioned the recently reviewed and developed strategies of standard means which include microscopic procedures, RDT or the rapid diagnostic tests, immunochromatographic tests, and molecular level identification, signified by systems based on PCR, additionally its limitations with recent trends on several new methodologies. These analyses provide practical comparisons, clarification and the feasibility between distinct diagnostic tests.

2.1 Routine laboratory diagnosis

Instant malaria diagnosis with productive outcomes reduces the probability of infection and minimizes community spreading as well. Hence, malaria is initially identified in the laboratory, employing different techniques. These procedures possess several advantages along with some shortcomings due to which recent modifications and new laboratory methodologies are being applied for future operations.

2.1.1 Blood smear as stained films

For malaria recognition, the laboratory procedure undertaken is the preparation with microscopic analysis of Giemsa, Field's or Wright's stained films of blood. Thick

and thin smear of blood are taken either by needling a finger or an earlobe. Evolved trophozoites are measured by their densities which are prominent in blood mainly in the regions where abundant capillaries are found; hence, it is the ideal specimen. Blood acquired by puncture of veins, gets accumulated in the covering of EDTA or heparin anticoagulant channel, is sustainable whenever it is utilized for the closure in the modification of the leukocytes structure along with the parasites [20, 21].

2.1.1.1 Blood film (thin)

Methanol and Giemsa or Wright's stain (diluted) is used to fix and stain respectively for the preparation of thin blood smear (film) and moderate water is employed at pH of 7.2. Since, in this technique, fixed RBC monolayer is obtained, parasite recognition by morphology is done at the stage of species and the particularity is raised in comparison to the study by the thick film. Further infections, where reactions for remedy can be examined, are done by the capability to add parasites in the sequential blood films.

2.1.1.2 Blood film (thick)

The red blood cells are centralized by the thick blood smear on the compact side and are stained as an immense composition by staining with Field, Wright or Giemsa techniques. This blood film method has very intense sensitivity as the blood elements are more concentrated. This is due to the thick smear of blood and is used for the observation of less quantity of parasitemia. It is vastly superior than the thin film with returning the passing on of parasites throughout the infectious phase restoration or degeneration [22].

2.1.1.3 Application example of microscopic inspection of blood smears

A comparative analysis was undertaken by Cortés et al., in 2018 of the technique originated and put forward by the world health organization, and employed for the pre-elimination programs and detection with cure of malaria in Colombia. The acceptable procedure was the thick film (two) composition which is stained by the improved Romanowsky stain in the same slide with its technical characteristics, of storage, low cost, usage along with a cure for the diagnosis of malaria which was from the consequences after the investigation [23].

2.1.2 Fluorescence microscopy

Substitute methodologies are established to intensify the blood film based recognition of malarial parasites. Specific dyes that are fluorescent have empathy for the genetic material present in the nucleus of the parasite that must affix in its nuclei. The nucleus becomes excessively luminant when UV light is used at a proper distance to excite it. Fluorochromes often used are acridine orange as well as benzothiocarboxypurine, both when energized gives fluorescence of yellow or apple green at 490 nm. Rhodamine-123 is beneficial in order to evaluate the parasite's feasible condition, when in consideration to the absorption that depends on the entire working parasitic membrane [24].

2.1.2.1 Acridine orange (AO)

AO is a staining technique which is directed or combined to an assembly practice like the thick smear of blood employed in numerous procedures. By employing a

particular long focal length objective accompanying a fluorescence microscope, the capillary tube can be helpful for the verification of the parasites. Within the passage of the transmitted beam of light an excitation filter is organized and enrolled by the simpler Kawamoto technique as well as permitting the affected wavelength of AO which is 470 to 490 nm, to move through the film stained. Additionally, in the ocular another filter of 510 nm has been set for making comprehensible AO stained fluorescing parasites observation.

2.1.2.2 Benzothiocarboxypurine (BCP)

BCP is another methodology utilized that can be put into a dry film of blood or in a suspension of lysed blood, unfixed along with profoundly staining the nucleic acid of feasible *P. falciparum* parasites. Inversely, the nuclei of leukocytes and RBC inclusions are not stained properly. The necessity of speedy investigation to halt precipitation of the dye is controlled by this activity, which was characteristic in some fluorescence systems. This element is noticed as a delicate and quick detection medium, similar to the staining by Giemsa stain, in various reports described mainly as *P. falciparum* having 95% sensitivity along with its specificity. Further, in the thick-film composition, the non-*P. falciparum* parasites are effortlessly recognizable compared to the QBC buffy coat. Workers having practice and performing these procedures where components with fluorochromes are involved can distinguish the parasites quickly and accurately.

Nowadays, fluorescent microscopes that are transportable and apply light emitting diode mechanization, glass slides that are pre-prepared as well as parasites labelling using fluorescent indicators, are accessible commercially [25].

2.1.3 Rapid diagnostic tests (non-microscopic)

Rapid diagnostic tests or RDTs are another very effective assay for the diagnosis of malaria which are contributing as a fascinating substitute for microscopy, detecting malaria parasite antigens and enzymes and forms the foundation of diagnosis in various impoverished areas where laboratory approaches are rare. Employing an immunochromatographic method, the RDT uses monoclonal antibodies to determine the antigen of malaria in 5 to 15 μL blood which is aimed at antigens specific utilizing a test strip for its permeation. The outcomes of these assays may be acquired in between 5 to 20 minutes that are elucidated from lines which are colored, present/absent on the strip. Aldolase (*P. falciparum* histidine rich protein II) and *Plasmodium* lactate dehydrogenase are identified by these tests. Considerably, for the differentiation of non-*falciparum* in between the *falciparum* infections, results from these procedures integrate any two among all the antigens for an attempt.The results from these test mechanisms are less accurate than outcomes obtained from microscopy achieved under routine field circumstances [26, 27].

2.1.4 Immunochromatographic assays

Immunochromatographic assays also known as Immunochromatography, over the surface of nitrocellulose membrane liquid, are relocated. In opposition to a target that is an antigen of malaria in conjugation with liposome constituting mobile phase with either gold particles or selenium dye, these methods got accepted with detainment of antigen from parasite obtained in peripheral blood and monoclonal antibodies are used in the entire process. During the immobile phase, a strip of nitrocellulose is taken where monoclonal antibodies are put in, which is the second/third capture. During the mobile phase, the antigen–antibody complex is

migrated alongside the strip, which permits the antigen labeled to record through the monoclonal antibody in the immobile phase constructing a colored visible line. Various physical features of the component reagents are crucial for migration and it is clear that the system is controlled for migration when a labeled goat anti mouse antibody capture is incorporated [28, 29].

2.1.5 Immunochromatographic dipstick assays

RDT dipstick format is another assay and is commercially accessible with its kits for the investigation of malarial antigens. In many laboratory investigations, comparison of immunochromatographic as well as conclusion acquired from PCR and also microscopic procedures has been examined. Even though some constructors of HRP-2 tests exist without the availability of published records, experiments for HRP-2 detection including ParaSight, ICT Pf or Pf/Pv together with PATH *falciparum* Malaria IC analysis was carried out on a large scale which are commercial dipstick trials.

For the *P. falciparum* identification in samples of blood, average sensitivity between 77% and 98% in attendance of 100 parasites/1micro liter with 0.002% parasitemia was resulted from experimental methodologies using ParaSight F immunochromatographic process of HRP-2. When compared with microscopic technique of thick blood smear, specificity between 83% and 98% for *P. falciparum* was obtained. The incapability of the viewer for the parasite exposure employing microscopic tests at densities reduced to 200 parasites/l or the failure of indistinct positive lines observation from the test strip is reflected by the insignificant level of sensitivity from multiple analysis [30, 31].

2.1.5.1 Application example of Rapid diagnostic tests

Speculative treatment with anti-malarial to all feverish patients gives rise to enormous over-treatment. Accordingly, it was directed to reduce drastically over-treatment with antimalarials on its prescription to evaluate malaria rapid diagnostic tests execution consequences at urban Tanzania, which was studied by D'Acremont *et al*., in 2011. Clinician experts with appropriate encouragement assemble with guidance of negative result patients for not treating them. Thus, to prevent unjustifiable use of antibiotics, execution of mRDT should be unified closely besides instructions for the control of various fever origins [27].

For the extraction of overall nucleic acid from operated mRDTs, Guirou *et al*. recently investigated and constructed and considerably estimated a technique. The observation along with the measurement of *P. falciparum* from patients with asymptomatic behavior by reverse transcription quantitative PCR which is allowed by the isolation of nucleic acids from low volumes of blood retained (dried) on the RDTs. Based on the procedure of separation they assembled Extraction of Nucleic Acids with RDTs procedure, an absolute advancement of extensive molecular malarial supervision. ENAR furnishes a strong mechanism to examine RNA and DNA from numerous RDTs in a high throughput and standardized way, disclosed during a review of malaria benchmarks employing RDTs collected. Finally, they got various novel and previously existed polymorphisms spreading in the Bioko Island of Equatorial Guinea, which were of single nucleotide and non-synonymous in the propeller zone of the kelch 13 gene [32].

2.1.6 Molecular diagnostic procedures

Molecular diagnostic techniques are the inspections where genetic material or the nucleic acids are being targeted and are verified as frequent, certain and acute

processes. Nucleic acid tests choose gene sequences like the *P. falciparum* STEVOR multigene family, 18 s ribosomal RNA gene sequences, telomere-associated repetitive component and mitochondrial DNA. Recent progresses in various molecular biological mechanisms including PCR and loop-mediated isothermal amplification (LAMP) have allowed considerable description of the malaria parasite which is bringing new approaches for malaria diagnosis.

2.1.6.1 PCR based methods

Recently, a number of PCR assays came into existence during malaria detection at a molecular level and among various other methods, it is manifested considerably as very sensitive and distinct techniques, particularly for low parasitemia or mixed infection examples. Beneficial knowledge is offered by multiplex and nested PCR mechanisms when demanding structural issues occur throughout the strive to recognize the species level parasites. A minimum of 1 to 5 parasites/μl blood approximated about 50 to 100 parasites/μl blood from RDT and microscopy can be recognized by PCR techniques. The species picked out is validated from the methods using reverse transcription PCR along with nested PCR. Parasites of malaria can be detected upto species level even in patients of minimum parasitemia level, and is the chief benefit of employing techniques that are PCR based. And these systems mostly investigate mutations and strain variation. Moreover, PCR can assist spot and examine parasite genes interrelated to mixed infections or drug resistance and also maximum numbers of specimens might be automated [33, 34].

2.1.6.2 LAMP technique

The Loop-mediated isothermal amplification (LAMP) technique is an additional nucleic acid method. This process has the possibilities of making adjustable strategies field based on nucleic acid, since low PCR with instrumentation is only needed and is effortless. Unlike PCR, amplification takes place at a constant temperature in LAMP and is an isothermal procedure.

In comparison to PCR, LAMP utilizes primers of three sets thus, challenging PCR in terms of quantity of DNA. The amplification can be determined in real time by checking the rise in the magnesium pyrophosphate quantity viz. turbidimetry. PCR is expensive and less sensitive than LAMP, which is also an uncomplicated approach, yet it has the downside for the necessity of a low temperature repository of reagents [35, 36].

2.1.6.3 Application example of PCR based methods

A study by Calderaro *et al*., in 2018 analyzed the current mode of imported malaria with revised inquiry as well as described the case results with their treatment, throughout January 2013 to June 2017, in Parma, Italy, with surveying the differentiation of the three indicative methods employed detection of malaria: microscopic techniques, Real-time PCR and immunochromatographic tests identifying, *Plasmodium malariae*, *Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium ovale curtisi and Plasmodium ovale wallikeri*. In their research, within the non-endemic areas, without multiple classic symptoms in different cases, the clinicians might get encouraged from the data described to doubt as malaria and to explain by parasitologists, the outcome of microscopic methods, other recommended procedures, and methodologies of molecular level to keep away from mis-detection [37].

A research from the Reference Laboratory for final malaria interpretation resembled the identification from laboratories of peripheral Belgian by Loomans *et al*., in 2019. The records of The Reference Laboratory for each specimen submitted in 2013–2017 were examined. As a result, very adequate malaria recognition mostly of *P. falciparum* was provided by the laboratories in a malaria non-endemic environment, with *P. falciparum* parasite density calculations and standard identification of non-*falciparum* infection [38].

2.2 Disadvantages and recent trends

The microscopic inspection in smears of blood, RDTs or rapid diagnostic tests with molecular or nucleic acid based methods consists of particular defects and restrictions in constructive utilization at environments where sufficient resources are not available. The enrichment and detachment without disrupting the parasite, which could not be acquired from these systems, might be crucial in malaria parasite study. To intensify the observation of iRBCs, further new practices were implemented by some research teams. Utilization of non-woven fabric size based filtration is one of the simple and inexpensive methodologies. Out of numerous other procedures, magnetophoretic with dielectrophoretic concepts are possessing pleasing prospects in the verification of malarial parasite recently, because of individual conversions with magnetic as well as electrical effects of hRBCs in contrast to iRBCs.

Furthermore, other procedures, in particular, deformability in addition to cell morphology, seemed acceptable for future use as biomarkers. An excessive magnetic field gradient can be generated by a Magnetic-field generating system throughout an immense region [39]. Next explanation is a mixture of more than two diagnostic assays, to raise the specificity to iRBCs, thus amplifying the capacity to detect and separate malaria and infected erythrocyte respectively.

3. Computational approaches against eradication of *P. falciparum*

Potential inhibitors against *P. falciparum* have been identified using different approaches like high-throughput screening (HTS) and drug discovery based on target approach. The HTS approach consists of identification of novel and effective inhibitors which facilitates the development of hits and validation of leads. The action mechanism of the leads, once validated, is identified. The advantage of this mechanism is that it helps to directly identify active compounds. Once the active compounds are successfully identified, the targets of only those compounds are validated. Another approach to drug discovery is target-based. This approach exploits the information of the target and thus, identifies new inhibitors using this information.

3.1 System biology approach

Establishing a target is a crucial part in drug discovery. In the past two decades few targets have been established as potential targets against antitubercular drugs. Target identification is mostly achieved by biochemical methods, genetic interaction or any other computational methods. In computational methods, target establishment involves gaining a detailed knowledge at atomic level along with study of structures of proteins, sequences of genes, pathways (metabolic) and protein–protein interactions (PPI) [40]. In a nutshell, finding a protein/receptor as a target, where a lead compound, a potent drug, may bind. Using the genetic data,

various techniques have been approved for searching a potent drug target. They can be summarized into four groups: *Gene-to-Target* method, *Disease-to-Target* method, Gene network and protein–protein interaction network.

3.1.1 Gene-to-target method

Initially, the common drug target classes were selected, later, computational techniques have been used to determine a new target and predict its function. Kinases protein, G-protein coupled receptors (GPCRs), the ions channel, and nuclear receptors are successfully established target classes.

After target class identification screening of possible new gene candidates is done from the sequence database. New gene candidates not only allow us in discovering new targets but also help us in understanding the disease at molecular level.

The human genome database contains a vast number of gene coding new proteins, thus, data mining the database can reveal new essential candidates of the target class. BLAST is the most commonly used technique for finding the similar sequence from the database. Sometimes more sophisticated approach viz., PRINTS are used. However, this limitation can be controlled using various *in-silico* techniques which include features like physiochemical properties, amino acid compositions etc.

The expressed sequence tags (ESTs) allow us resources for characterization of gene, identification of new gene and expression of gene with specific tissue. The EST database (dbEST) not only helps in identifying new genes of the class target but also expression of gene Wittenberger *et al*. illustrated an EST database method to search for a new member of the superfamily, GPCR. They have found about 14 new members as new putative for GPCRs [41].

Gene function identification is one of the most essential parts for drug discovery. Function prediction is proved to be a difficult task, even species like *Escherichia coli* contain about ~30% genes whose-of gene's functions are still unknown. Similarly, in *Plasmodium falciparum* about ~60% of the genes are lacking their function [42]. The approaches for gene function prediction are still way back than lagging behind as compared to the progress that has been made in genome sequencing [43].

3.1.2 Disease-to-target method

Identifying a target requires the study of the origin of the disease and their relation with the system. This method initially focuses on one specific disease and later on various approaches like expression of gene and analysis of linkage are acquired for identifying disease related genes along with potent targets for drugs.

Hundreds of gene expressions can be generated and utilized to identify disease related genes and potent target thorough microarray. Microarray techniques, apart from identifying gene targets and pathways, also allow us to understand the biological process of the disease. Identification of the target can be done by understanding how a potent drug or compound influences a particular metabolic pathway or regulates a network in cellular metabolism.

Predicted disease models that are used during experimentation can be used for validating a target. Thus, a disease model at the molecular level predicts the compounds that are best against the disease and also to perform the binding of the ligand through *in-silico*. Pettipher *et al*. have used this approach to find out inflammatory diseases associated with GPCRs. This leads to identification of new targets associated with genetics [44].

3.1.3 Gene network method

This method deals with the modeling of signaling, endogenous metabolic and regulatory networks along with potent drugs interacting with targets. This helps in recognizing if a target is inhibited and it resulted in inhibition of other activities which are in relation with that pathway. The interactions of genes are essential for studying the structure, functions and the organism's response. There is a possibility of deducing the gene network predictive model by the over expression of the gene present in the network and calculating the overall expression result of the entire gene during a stable state in the gene network. Simulation and analysis through mathematics can make a successful prediction. Various computational approaches have been approved as equation models, Bayesian networks and Boolean networks for deducing regulatory gene networks.

3.1.4 Protein–protein interactions (PPIs) network

In the drug discovery process proteins act as the main target. The expression of protein on both the normal and the diseased one plays a crucial role in drug targets and drugs development. PPI network is one of the methods for identifying essential drug targets. The interaction map of protein shows the important pathways which can be studied for target identification. They are further validated in the biological model. Although there are hundreds of pathways associated with a disease metabolism, the main role is picking out an essential node in the complex gene or protein network which can be utilized as a potent target [45]. The graphical representation of the pathway can estimate the overall topology and target node but the behavior of cell response is quite limited. To overcome this kinetic model approach was adapted.

In the static network method the constructed network is analyzed using various topological analyses which can be related to various functions and regulation mechanisms. Further a constructed static network can be compared with a well-known network thus, revealing various unknown biological functions of the system [46]. Some examples are Cytoscape, String and Gephi.

Kinetic model is also known as the mathematical approach used to determine the dynamics of the biological system. The model can be validated under various environments. The kinetic model uses some of the reaction parameters viz. Michaelis constant (K_m), turnover number (K_{cat}) and enzyme concentration (E_0). In absence of experimentally estimated parameters, parametric estimation methods and Monte Carlo are used [47].

In this context, Bora *et al*. have selected about 39 proteins out of which 15 proteins were screened out for further studies. This protein's interaction was retrieved from the STRING database. The filtered proteins along with their interacting protein are subjected to cytoscape to create a network. Further for mutant modeling and structure analysis the Adenylosuccinate lyase (ADSL) is selected. The docking resulted in ADSL complex, docked with AMP. MD simulation is performed for stability analysis [48]. Bora *et al*. have performed a computational study on *Leishmania donovani*, integrating PPI with metabolic pathways. Purine salvage pathway was considered for the study and the overall biochemical reactions were taken from the KEGG database, then the kinetic model was subjected to simulation in "COPASI". Finally, the PPI of *L. donovani* is derived from "STRING version 10.5" which was analyzed in Cytoscape using the cytohubba plugin [49].

3.1.4.1 Application examples of target identification against P. falciparum using systems biology approach

P. falciparum PPIs were identified using high-throughput kind of yeast-two hybrid assay. LaCount *et* al. identified about 2,846 new interactions of which most of them were uncharacterized proteins. Further, the *in-silico* analysis of PPIs connectivity of the network, gene expression which are coding for interacting segments and Gene Ontology were done to identify protein interacting with mRNA, transcription, modification of chromatin and those taking part in the invasion of the host cell [50]. Proteins having topology associated with significant graphs and high degree were considered as drug targets.

Saha *et al.* have made a case study of pathogens (*P. falciparum*) with host (Human). PPIs of both interacting with each other are derived from the STRING database. The node weight and edge weight has been computed simultaneously. Protein's centrality and connectedness values play a crucial role in organization of function. The node and network centrality identifies the most important protein in the network. Around 457 new interactions were identified of which 198 bait proteins and 217 prey proteins were mapped to the STRING database. Further, PANTHER was used for the analysis of functional similarity [51].

3.2 Bioinformatics approach

Bioinformatics approach facilitates identification of targets for drugs, drug molecules and screening of compounds. In addition to this it also scrutinized the side effects along with prediction of resistance of drugs. It covers almost every aspect of mechanism drug discovery through data of high throughput [52]. Apart from this homology modeling and protein simulation covers the field for more vivid virtual screening and docking studies.

3.2.1 Protein structure prediction

Tertiary structure of protein is the most essential requirement for studying the folding, unfolding along with the function of a protein. But determining the structure is not always easy. X-ray crystallography, nuclear magnetic resonance (NMR spectroscopy) and Cryo-electron microscopy (Cryo-EM) are the major techniques used for prediction of the protein 3D structure. But each of them has its own limitation keeping aside the cost part. In the past few decades computational techniques have developed quite a lot. Predicting the 3D structure not only allows studying the folding and unfolding of the protein but also may facilitate new function in a protein. The tertiary structure of proteins is mainly achieved in three ways:

3.2.1.1 Based on empirical energy calculations

Energy minimization, also known as *ab-initio,* is a way of predicting protein structure. The structure is established by the minimum energy of a protein structure at thermodynamics equilibrium. Rather than making an account on the theory of the amino acid properties it aims in locating the protein's free energy global minima as it resembles the conformation of the naive protein. Some of the essential force fields used are CHARMM, GROMOS, AMBER, ECEPP, and ROSETTA-ab initio.

3.2.1.2 Based on experimentally determined 3D structure Knowledge approaches

Knowledge based approaches are another way of predicting the 3D structures. Currently, comparative modeling or better known as homology modeling is the most common and powerful technique utilized for predicting the tertiary structure of proteins. It compares the query sequence of protein with a similar sequence to that of a known crystal structure. Thus, a precise model can be established based on sequence similarities along with building the model and energy minimization. MODELER and Distance geometry are the most commonly used analysis tools.

Another approach is the threading which aims for selecting the most possible fold of the query sequence or recognizing the possible sequences which fold into the structure. Recognizing a fold needs the perfect alignment of query sequence to that of a folding motif's amino acids. Precise structure can be designed only if atomic level details of the folds are available [53].

3.2.1.3 Hierarchical methods

The last alternate approach for predicting protein structure is the Hierarchical approach which uses the overall hierarchy, i.e., primary to tertiary structure. Here the prediction of the secondary structure is done for understanding the association between the primary sequence and the 3D structure. Few methods based on physicochemical feature approach, statistical approach, combinatorial approach, information on evolution and artificial intelligence approach have been adopted for predicting the secondary structure. Even though, using these algorithms the accuracy rate is still at 80% [54].

Intensive study of chloroquine (CQ) drug resistance in *P. falciparum* identified transporter protein (*Pf*CRT) association with the resistance. Antony *et al.* modeled both the wild and mutant type of *Pf*CRT 3D structure in Robetta and I-TASSER using threading [53]. Similar work has been performed by Sanasam and Kumar. An essential protein of *P. falciparum* AMR1 has been identified as a potential vaccine candidate. They predicted the 3D structure of AMR1 in ROSETTA using an *ab-initio* approach. Further, they predicted the best B-cell and T-helper epitopes of the protein [55]. Borah *et al.* analyzed the sequence of HopS2 protein which revealed many essential regions of the protein secondary structure. The target is then subjected to homology modeling in Swiss model but for lack of low coverage of the sequence the target has been modeled using an *ab-initio* approach. MD simulation in 100 ns has validated the stability of the target protein [56].

3.2.2 Computer-aided drug design approaches

Experimentally screening thousands of compounds along with their targets is quite laborious as well as time consuming too. As a result, *in-silico* methods were adopted, to identify the perfect hits and also improvise the probability of finding a precise drug molecule. For initiating drug discovery and design, various techniques are available, which are mostly: ligand-based (LBDD) and structure-based (SBDD) drug designing. LBDD approach involves methods such as pharmacophore modeling and quantitative structure–activity relationships (QSAR) whereas the SBDD approach involves methods such as molecular docking and molecular dynamics simulation.

3.2.2.1 Quantitative structure–activity relationships (QSAR)

Development of a quantitative structure–activity relationship equation is entirely based on the assumption that the molecular structure can be quantitatively

correlated with the molecule's physicochemical or biological activity. Thus, the working concept behind this methodology is the differences observed in the biological activity of a series of compounds which can be quantitatively correlated with the differences in their molecular structure [57]. The final outcome of a logical and mathematical procedure is known as the molecular descriptor and it should be able to transform the chemical information, encoded in a molecular symbolic representation [58].

Some of the commonly used descriptors are the **hydrophobic descriptors** which represent the whole molecule size or lipophilicity [59] using properties like the molecular weight, volume and logarithm of partition coefficient in 1-octanol and water (log P), etc. **Steric descriptors** are those which explain both the shape and size of the molecules and the substituents. Some commonly used steric descriptors are the Taft's steric constant Es, molar refractivity (MR), surface areas, molecular volume, etc. **Topological descriptors** are obtained from hydrogen-depleted molecular graphs. Wiener index, structural information content, Chi index, molecular similarity, electro-topological state atom (ETSA) indices and Kappa index, etc. are some of the commonly used Topological descriptors [60]. **Electronic descriptors** are those that can justify the electronic properties of a molecule and are represented by descriptors such as, the Hammett constant (σ, σ-, σ+), charge transfer constants, dipole moments, ionization constant pKa, ΔpKa parameters derived from molecular spectroscopy and parameters derived from quantum chemical calculation. The quantum chemical descriptors based on **density functional theory (DFT)** and semi-empirical methods have been found to play a major role in QSAR studies. Some of the commonly used DFT-based descriptors are the chemical potential (μ), global hardness (η), global softness, electrophilicity index (ω), etc. The study for artemisinin (an important class of antimalarial drugs) and its derivatives using the DFT based descriptors confirmed that one of the molecules (molecule-13) was the most reactive considering the electronic configuration [61].

3.2.2.2 Pharmacophore modeling

Pharmacophore modeling is the assembling of all the frameworks of a particular molecule which are necessary features for a molecule to inhibit a target's biological response. Over the course of time this methodology has become crucial tools in drug discovery and designing. Designing a model can be accomplished by using **ligand based** where the active molecules are superimposed and similar electronic and steric features of importance are considered and **structure based** where the interaction between the target and the ligand are explored [62]. Manhas *et al.* did a pharmacophore study where they attempted to identify novel inhibition against *P. falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) and also utilized docking and MD [63].

3.2.2.3 Molecular docking

SBDD is based on the target and the ligand's 3D data whereas LBDD can be implied in absence of targets 3D structure. Here, the ligands were known which remained bound with the targets. Further, the targets were analyzed to decrypt the structural as well as chemical properties of the ligands. Molecular docking is divided into three types: flexible, semi-flexible and rigid docking. During the past decades, different types of approaches have been made like conformational sampling and induced fit models [64, 65]. Some examples are: GBVI/WSA, LUDI, Monte Carlo, GoldScore and AutoDock.

Hazarika and Jha have considered three different types of docking programs viz. Molegro Virtual Docker, Glide and GOLD, a set of ligands screened out based on the druglikeness properties are considered for docking against the target protein influenza endonuclease, RNA binding protein. The resulting target-ligand complexes lists are considered based on their scoring functions. Among the top 20 compounds, 10 compounds were common in the mentioned software. Thus, indicating some consent of the compounds [66]. Thillainayagam *et al.* carried out an *in-silico* study by screening the set of pyrazole-pyrazoline against the target Histo aspartic protease (HAP). The target is docked with the set of the ligand (Asp 215, His 32 and Ser 35) as their binding site in Auto dock version 4.2. Further, MD simulation revealed HAP-ligand as more stable than HAP [67].

3.2.2.4 Molecular dynamics

Molecular dynamics (MD) simulation technique allowed us to understand the time-dependent behavior of protein-ligand interactions. MD simulation mimics the moment of an atom; the fundamental unit of a molecule, and what it does in real life. In MD simulation, which is based on Newtonian mechanics, forces on each atom are computed provided we know the energy function and positions of the atoms in a system. Generally, 2 fs (femtosecond) is applied for simulating the atom which is integrated with the numerical movement. The common and popular engines available are: GROMACS, DESMOND, CHARMM, NAMD and AMBER. Their integration with message passing interface (MPI) has significantly reduced the overall computational time by making the engines utilize the cores simultaneously [68].

Hazarika and Jha have utilized molecular dynamics in measuring the interactivity of silver nanoparticles on human serum albumin (HSA), a transport protein. Sudden change in its structure can barricade the overall function. The HSA sequence analysis was done using BLASTP and MSA followed by the secondary structure analysis of HSA. Further, MD simulation was performed in GROMACS 2016.4 version for checking the functionality of the nanoparticles with HSA [69]. In order to effectively challenge Epstein-Bar virus, Jakhmola *et al*. have applied molecular docking and MD simulation of glycoprotein H with different phytochemicals and showed their potentials as virus specific therapeutics [70]. Saikia *et al*. performed MD along with molecular docking to check the dynamics of pyrazinamide (PZA) and PZA-functionalized graphene interaction with pncA protein. A total of five sets of systems were subjected to MD simulation. Using Gromacs package 4.5.0 along with OPLS-AA force-field MD was performed [71].

3.2.3 Application examples of drug discovery against P. falciparum using Bioinformatics Approach

P. falciparum falcipains act on hemoglobin degradation, making it a potential target for drugs. Musyoka *et al.* performed an *in-silico* study on falcipain-2, falcipain-3 and their homologs. Using MODELER 9.10 homology models were prepared and docking was done in Autodock 4.2 using the 23 natural compounds against 11 target proteins. Based on docking energies 3D similar search is performed in ZINC. Further, MD simulation confirms the interaction of the derivatives with the targets. Overall they provided a more effective derivative of the compounds, making it well tolerated among humans [72].

Phosphatidylinositol-4-OH kinase (PI(4)K) type IIIβ, a type of lipid kinase has been recognized as an essential imidazopyrazines target. Unavailability of crystal structure posed a challenge for drug development. So, Rajkhowa *et al.* performed an *in-silico* where they utilized homology modeling to model the *Pf*PI(4)KIIIβ 3D

structure. And they validated the structure using MD simulations. Moreover, taking the toxicity and hERG liabilities as priorities screening was done for 178 compounds from PubChem database. The ligand and target were subjected to molecular docking in AutoDock 4.2. MD simulation was further utilized against the target-ligand complexes to check stability. The study reported a total of three inhibitors based on the analysis done [73].

4. Conclusion

Malaria, the world's most regular and dreadful tropical disease, considerable actions have been undertaken to arrest or cure malaria from several decades. Diverse *in silico* attempts including high throughput screening that has screened out a number of novel drug molecules like phytochemicals which can be a potent antimalarial drug in near future, antimalarial target determination, modeling of target proteins using homology modeling, Pharmacophore modeling of derivatives in the existing resistant drugs, Ligand and structure based drug designing strategies for the development of potent drugs, along with several experimental techniques. However, it provides sensitivity along with specificity that varies resembling recent technical advances. RDTs are convenient and currently implemented in many remote settings but could lead to overdiagnosis and overtreatment, demanding upgraded quality control. Molecular-biological methodologies put forward the best sensitivities, they can be utilized to identify the development of drug-resistance, are useful for species identification, and also for quantifying parasite density with low parasitemia. However, out of reach to most and are restricted to clinical research. There are various possibilities and a wide range of applications of CRISPR/Cas9 systems evolved in prokaryotes. Using this technique, gene drives to make mosquitoes resistant to malaria have already been planned and further study can be carried out with proper examinations. Also genetically engineered mosquitoes can be developed that produces almost entirely male offspring, consequently helping in malaria eradication. To lessen the parasite would call diagnostic tools which are non-invasive highly sensitive, vigorous and go with the environment established in particular regions.

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