Malaria is one of the prevalent vector-borne diseases in India. Though it is a curable disease, failure to recognize it early and increasing resistance to present antimalarial drugs are the major obstacles in its treatment. Thus, irrespective of substantial advancement, there is a demand of supplemental diagnostic methods with high sensitivity and specificity to account for early diagnosis and treatment. Additionally, advanced antimalarial drugs and profound research in pathophysiological mechanism of antimalarial drug resistance is also indispensable. In this review, we have summed up the presentations on malaria that were presented during the 40th Annual Conference of Indian Association of Medical Microbiologists, MICROCON 2016 at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, from November 23 to 27, 2016. Videos of the talks held in the conference are also available on website http://microcon2016.com/conference-videos/. Major talks were focused on the development of newer diagnostic techniques with high sensitivity and specificity. Further, research on the markers for drug resistance and prevention of spread of drug resistance in malaria was also discussed closely.

**Key words:** Diagnostic methods, Malaria, Markers for drug resistance.

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**REVIEW**

Malaria is one of the major public health problems worldwide with 3.2 billion people are at risk of getting malaria. In 2015, 214 million cases of malaria were reported and 43,800 deaths occurred. About 88% of the total world malaria case was recorded from African region in 2015, 10% from Southeast Asia region, and remaining 2% cases were from Eastern Mediterranean region. Maximum deaths (90%) because of malaria were reported from African region, followed by 7% in Southeast Asia and 2% in Eastern Mediterranean region. Malaria is also one of the main causes of deaths in 5-year-old children in sub-Saharan Africa.¹

**Malaria Research in MICROCON 2016**

The 40th Annual Conference of Indian Association of Medical Microbiologists, MICROCON 2016 was scheduled at PGIMER, Chandigarh, India, from November 23 to 27, 2016. The conference covered wide range of topics from the fields of medical microbiology, parasitology, and virology with 1,436 delegates, 135 faculty members including 16 international faculties as participants. Majority of Indian centers that are engaged in research, teaching in the above fields, and providing services to patients were the prominent part of conference. The present review covers the talks and presentation related to malaria at the conference.

The conference commenced on November 23, 2016 with a remarkable workshop on “Malaria: Advances in Diagnosis and Drug Susceptibility Testing.” The presentations on recent advances in the diagnosis of malaria, drug resistance, and drug susceptibility testing in malaria were delivered in detail. Further, contributor delegates were demonstrated with various diagnosis techniques of malaria. These techniques included nested polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), gel electrophoresis, culture, PCR-restriction fragment length polymorphism (RFLP), and sequence-based detection of drug resistance. On November 24, 2016 preconference continuing medical education (CME) was scheduled in the Bhargava Auditorium of PGI that covered presentations on “Rapid and Point of Care Tests.” On 26 November, various presentations on “Malaria: emerging issues” were delivered at the same venue. The conference covered 10 oral presentations on malaria by speakers from various esteemed institutions of India.

**Recent Advances in the Diagnosis of Malaria**

Diagnosis of malaria can be made by employing various techniques including clinical diagnosis, microscopic diagnosis, serology, and molecular diagnosis. Majority of the diagnostic techniques used presently require high skilled staff, costly and delicate equipment, and electricity supply, failing which it may lead to false-positive results and misdiagnosis of malaria. The errors in malaria diagnosis can expose patients to unwanted antimalarial drug resistance.
therapy and probably lead to drug resistance. These issues of false results and overtreatment of malaria need to be controlled with the development of more trustworthy, field-suitable, and inexpensive diagnostic tools. The areas belonging to malaria pre-elimination settings need highly sensitive diagnostic tools for field studies for the detection of residual malarial infection. Very low parasitemia often fails to get diagnosed as it remains below the limit of both microscopy and rapid diagnostic tests (RDTs), which accounts for highly sensitive methods like PCR and LAMP to be used. A recent study by Aydin-Schmidt et al evaluated the performance of high-throughput LAMP against real-time PCR on 3,088 samples in a community-based survey. The study concluded that though high-throughput LAMP is field applicable, it is insensitive to be used for the detection of asymptomatic low-density infections in areas approaching malaria elimination.

In a study by Piera et al, LAMP was found to be highly sensitive for field studies in the detection of Plasmodium knowlesi infection. According to this study, there are many reported shortcomings of microscopy and RDTs in the diagnosis of *P. knowlesi* infection, which can be overcome by the use of LAMP for field studies.

In the workshop on malaria scheduled in MICROCON 2016, Dr Abhishek Mewara, Assistant Professor, Department of Medical Parasitology, PGIMER, Chandigarh, India, delivered presentation on “Recent Advances in the Diagnosis of Malaria.” He focused on nucleic acid amplification techniques with real-time PCR, LAMP, and its modifications. Recent advancement in these methods allows detecting even very low count of parasites in the blood sample. It was concluded that LAMP is a highly sensitive and suitable method to be used for field operations that can detect infections in patients with low parasitemia missed by conventional microscopy.

Various studies have been reported recently to compare the sensitivity and specificity of different diagnostic methods of malaria including quantitative buffy-coat (QBC), microscopy, RDT, and PCR. Ms Nonika Rajkumari of Jawaharlal Institute of Postgraduate Medical Education & Research (IIPMER), Puducherry, India, presented a similar comparative study in the oral paper presentations session of conference entitled “Discrepancies in the routine diagnostic methods of malaria.” Hundred patients suspected of malaria were included in the study and their blood sample was taken before treatment and after 1 week of treatment. These samples were tested with isochromatographic test (ICT), thin and thick smears, QBC, and PCR. Seventy samples were positive by thin and thick smears, 74, 91, and 199 by QBC, ICT, and PCR respectively. It was found that some samples which were undetected by thick and thin smear showed positive result by ICT and PCR. It concludes that in case of partially treated patients with malaria where parasite load is low, a combination of tests should be used to diagnose the disease.

Ms Sreemanti Debnath from the Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sevagram, Wardha, Maharashtra, India, also displayed an oral presentation based on the comparison of different diagnostic techniques. She described the diagnostic performance of microscopy, RDT, and PCR in the diagnosis of *Plasmodium falciparum* malaria. Out of 200 samples, magnitude of the disease was found to be 16.9%. Sensitivity and specificity of microscopy, RDT, and PCR using latent class analysis were found to be 67.8 and 100%, 91.3 and 98.2%, and 78.3 and 93.7% respectively. This study also indicated that RDT was the best option for the detection of malaria in resource-limited areas.

### Drug Resistance in Malaria and Drug Susceptibility Testing

Drug resistance in malaria is defined as “the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject.” The drug must gain access to the parasite or the infected red blood cell (RBC) for the duration of the time necessary for its normal action.

Drug resistance is the major reason behind the failure of malaria elimination goal. Though various drugs show resistance against *P. falciparum*, resistance against *Plasmodium vivax* has also been reported in various areas. Chloroquine (CQ) resistance in *P. vivax* has been found in major areas of India, Burma, Indonesia, Papua, Colombia, New Guinea, Brazil, Guyana, and Solomon Islands.

Chloroquine resistance in *P. falciparum* is due to the gene *pfmdr*-1 and *pfmdr*-2 and *pfcr*, which was first reported in 1973 in Assam, India. It is severe in north-east and southeastern regions of India. Sulfadoxine/pyrimethamine resistance in *P. falciparum* is due to the dehydrofolate reductase enzymes, which was first reported in Thai Cambodian border in the 1960s and nowadays mostly reported from Southeast Asia and the Amazon Basin. Quinine resistance in *P. falciparum* is due to *pfmdr*-1 mutation, which was also firstly reported from Thai Cambodian border in the mid-1960s; nowadays it is being reported from Southeast Asia and in India from Kolar district in Karnataka and northeastern states. Mefloquine resistance in *P. falciparum* is due to polymorphism of *pfmdr*-1 gene with prominent cases found in Southeast Asia, Colombia, New Guinea, Brazil, Guyana, and Solomon Islands.
Advances in Malaria at MICROCON 2016

Chemoprophylaxis is recommended for the travelers visiting malaria endemic areas. It should begin a week before arrival in the malarial area and continued for at least 4 weeks after leaving a malarial area.12

Recent data survey reveal that in a study conducted by National Vector Borne Disease Control Programme during 2003 to 2006, 36 to 95% of CQ treatment failure was reported in \textit{P. falciparum}. A three times increased incidence of CQ resistance was also reported during 1995 to 2002.13

There are distinct ways used to prevent drug resistance like careful selective use of drugs; improving the usage practice of drugs (betterment in follow-up practices and patient conformity); or using drug combinations and facilitate development of spread of resistant parasites.14

In the workshop, Dr Kapil Goyal, Assistant Professor, Department of Virology, Postgraduate Institute of Medical Education & Research, Chandigarh, displayed presentation on “Drug resistance in malaria and drug susceptibility testing.” It was pointed that due to high rate of drug resistance cases, presumptive treatment of malaria has been banned and it is mandatory to start treatment only after confirmation of infection. Mono-therapy with artemisinin as treatment of malaria is also banned by the recent guidelines on treatment of malaria issued by the Government of India.15 As per National Antimalarial drug policy in India, artemisinin is recommended in combination therapy along with sulfadoxine/pyrimethamine.1 The main reason behind the increasing number of resistant patients was the easy availability of artemisinin and its use as a monotherapy, poor quality of drugs, and consistent presence of mosquito breeding sites at Thai Cambodia border. It was concluded that Kelch 13 mutations are involved in artemisinin resistance.16 The ring survival assays have been developed to check \textit{in vitro} drug susceptibility and molecular assays are being developed to analyze drug resistance.

Dr Rakesh Sehgal, Professor and Head, Department of Medical Parasitology, PGIMER, Chandigarh, also gave presentation on status of drug resistance in malaria during CME of the conference. He discussed that two separate studies by Witkowski et al17 and Amato et al18 derived a molecular marker for piperaquine resistance, \textit{plasmepsin 2} and \textit{plasmepsin 3} gene amplifications on chromosome 14 in Greater Mekong subregion of Cambodia.

Amato et al18 also identified an additional marker, an exonuclease gene polymorphism on chromosome 13 (exo-E415G), in strong linkage disequilibrium with \textit{plasmepsin 2} to 3 amplification. These studies make \textit{plasmepsin 2} to 3 amplification a robust marker for piperaquine resistance in the region. Once resistance to both artemisinin combination therapy components appears, selection of multidrug resistant parasites will strongly amplify because of high treatment failure rates and higher gametocytemia in recrudescence infections, driving transmission to the \textit{Anopheles} species vector.

A case report was also presented in the talk, which describes that dihydroartemisinin–piperaquine (DHA–PPQ) treatment failed for a \textit{P. falciparum} case in Italy. This patient had visited Ethiopia. This patient had a recrudescence of \textit{P. falciparum} malaria after 32 days of DHA–PPQ treatment.

Piperaquine resistance is strongly associated with amplification of plasmepsin 2 to 3, encoding hemoglobin-digesting proteases, regardless of the location. Multicopy plasmepsin 2 constitutes a surrogate molecular marker to track piperaquine resistance. A molecular toolkit combining plasmepsin 2 with \textit{K13} and \textit{mdr1} monitoring should provide timely information for antimalarial treatment and containment policies.17,18

Ms Lipika Barman from Assam Medical College presented work on molecular detection of drug-resistant \textit{P. falciparum} mutants in northeast India. In this study the prevalence of \textit{PfK76T} and \textit{Pfmdr-1-N86Y} point mutations with CQ resistance and MAL-10, MAL-13, and \textit{Kelch} 13 propeller gene for artemisinin resistance in northeast Indian \textit{P. falciparum} clinical isolates was checked. Prevalence of \textit{PfK76T} mutation was found in 96.66% cases and \textit{Pfmdr-1-N86Y} mutation was found in 84.21% cases. They found a mutation in Kelch 13 gene sequence in relation to artemisinin resistance. There was no mutation detected in MAL-10 and MAL-13 genes.

Ms Hargobinder Kaur from PGIMER Chandigarh presented work on prevalence of polymorphisms in antifolate and CQ drug resistance molecular marker genes \textit{Pdhdr}, \textit{Pdhips}, \textit{PdcoRt}, and \textit{Pdmdr-1} in clinical isolates of \textit{P. vivax} in Chandigarh and adjoining states. Variable number of GGDN repeats in \textit{Pdhdr} has been found ranging from two repeats (17%) to four repeats (3%). The double mutant haplotype S58R+S117N was seen in 21% of the clinical isolates. Only 9.6% had single haplotype D459A and 1% of the clinical isolates had the mutant haplotype A553G in \textit{Pdhips} gene associated with drug resistance to sulfadoxine drug. None of the clinical isolates showed the insertion of the lysine in the first exon in the \textit{PdcoRT} gene. In case of the \textit{Pdmdr-1} gene all the samples showed wild-type allele at the codon 976 and 1074. The present studies concluded that the clinical isolates are still susceptible to CQ therapy as compared with antifolate drugs.

Anantabotla Vamsi Mohan of JIPMER Puducherry and Dr Subhash Chandra Parija, Director, JIPMER, presented work on genotyping of polymorphic marker \textit{MSP3β} gene of \textit{P. vivax} isolates from Puducherry and Mangaluru. \textit{PvMSP3β} proved to be a reliable polymorphic marker.
for population genetic analysis of *P. vivax*. Also, PCR/ RFLP was found to be a powerful tool for genotyping and identification of mixed parasite infections without the need for gene sequencing.

Ujjala Ghoshal from Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India, delivered presentation on identification of mutations in *P.fndr-1*, *Pfpt-6*, *Pemdr-1*, and *Pocrt(o)* genes in *P. falciparum* and *P. vivax* from Northern India.

Dr Shirish Malvankar, Consultant, Diagnostic Product Development and Director Laboratory Operations at Pyramid Referral Laboratory (P) Ltd and Lilac Medicare (P) Ltd, presented “Paradigm shift in Malaria Diagnosis.” The presentation was focused on the development of techniques related to diagnosis of malaria.

Currently malaria is diagnosed both microscopically and nonmicroscopically. Microscopy is the gold standard for the diagnosis but it is time consuming and the sensitivity is also compromised if the quality of stains used is substandard, technical expertise is poor, or the level of parasitemia is low in the blood sample. Light microscopy and fluorescence microscopy are the two types of microscopic techniques available. Light microscopy is still widely used to diagnose malaria as fluorescence microscopy is comparatively expensive. Fluorescence microscopy is less time consuming but its sensitivity is compromised by the nonspecific nature of fluorochromes for malaria parasite. Further, skill of the highest nature is required to detect malarial parasites on this microscope.

The nonmicroscopic methods of diagnosis of malaria include rapid card tests, QBC, PCR, digital cytometry, and enzyme-linked immunosorbent assay. Rapid card tests are qualitative tests because it differentiates *P. vivax* and *P. falciparum* species. Low sensitivity of RDTs makes it difficult to detect parasitemia less than 100 parasites per microliter. Rapid card tests are good as point-of-care test (POCT) but these tests provide partial diagnosis because these do not confirm the severity of infection. Rapid diagnostic tests are also not good as prognostic marker since enzymes/proteins persist long after the clearance of infection. So molecular techniques like PCR are widely used as they are more sensitive and can detect even 1 to 5 parasites per microliter. Polymerase chain reaction techniques can also differentiate the various malarial species but is an expensive test.

It was further discussed that there is a need of a diagnostic method which detects malarial parasites at a very low parasitemia, accurately speciates the malarial parasites, reports actual counts of malarial parasites, removes subjectivity of a microscopist, uses standardized specimen preparation, and at a reduced and affordable price.

Sight Diagnostics (Israel-based medical diagnostic company) has introduced a new diagnostic technique “ParaSight Digital cytometry.” This digital cytometry machine provides a unique cartridge for monolayer preparation of sample causing no distortion of cells as in peripheral smears. Even cell distribution causes easy scanning of all the cells. There is advanced tricolor high-resolution imaging, i.e., 1 bright field and 2 fluorescent images per field. This unique two fluorochrome signal helps classify cells. This technology provides novel algorithmic parasite identification, i.e., it characterizes cells based on multiple measurements (cytometry) like size, shape, density, granularity, and fluorescence. In this digital cytometry, total time taken to prepare the sample is 15 seconds/sample and scanning and analysis time is 4 to 5 min/sample.

There are many advantages of digital cytometry in the diagnosis of malaria over other methods like high sensitivity and specificity (over 98%), detects low parasitemia (<20 parasites/µL), has accurate speciation (over 98%) for all species including mixed infection. It gives exact parasitic index, high throughput (1–30 samples per batch). It is fully automated, so minimal training is required for the person handling and offers affordable cost per test.

Dr Kavita Singh, Program Director of Multivaccines Development Program, New Delhi, India, delivered the talk on “Development of Point of Care tests (POCT).” The POCT can lead to rapid diagnosis of malaria. However, POCT should have qualities like affordable price, sensitive, specific, user friendly, rapid and robust, and equipment free and give results quickly.
Various technologies used in POCT consist of microscopy, lateral flow immunoassay, and nucleic acid amplification test. Malaria is mainly diagnosed by stained slide using microscopy, antigen/antibody detection using RDTs, or by ELISA and molecular detection by PCR.

Table 1 compares various diagnostic techniques of malaria. Newer advanced microscopes, such as cell scopes can give results in minimum time with high sensitivity.

In microscopy, the development of digital imaging technology and artificial intelligence can diagnose parasites very clearly by a system of pattern recognition.

A new technique based on lab on chip is available now in which sample preparation and analysis can be done on the same small chip. Cell microarray chip has been used in Uganda for the diagnosis of malaria. In this technique, plasmodium-infected RBCs are stained by a specific fluorescent dye and are detected by fluorescent detector.

The current advanced techniques like phone-based devices and nucleic acid amplification test-based devices are costly but are outstanding in giving better results.

*Plasmodium knowlesi* is the recently discovered fifth human malaria. *Plasmodium knowlesi* usually causes infection in macaque monkeys but in recent times it has been reported to cause infections in humans, mainly in the Southeast Asian regions. *Plasmodium knowlesi* was first described in the early 1930s in a *Macaca fascicularis* specimen from Singapore. In humans it was first reported in 1965 in an American man who had returned after working in the jungle in peninsular Malaysia. In 1965 the first human case of *P. knowlesi* was detected in Peninsular Malaysia. An American-based man was found infected with it who was working in Peninsular Malaysian jungles before returning to America. According to a review published in 2011 by Anu Kantele and T Sakari Jokiranta, hundreds of *P. knowlesi* cases have been reported from Southeast Asian countries including Myanmar, Malaysia, Thailand, Singapore, Philippines, Indonesia, and Vietnam.

Dr Sumeeta Khurana of the Department of Medical Parasitology, PGIMER, delivered a talk on *P. knowlesi*. The pathology of *P. knowlesi* resembles that of *P. falciparum* and it causes very severe disease. The morphology of *P. knowlesi* resembles that of *P. malariae*. In some cases the *P. knowlesi* infection is misdiagnosed as *P. malariae* and is thought to cause mild disease, which leads to complications.

### Changing Clinical Features of *P. vivax* Malaria

Major burden of malaria infection worldwide is mainly due to *P. falciparum* infection. However, *P. vivax* malaria is also a big challenge as 40% of the world population is at its risk. About 2.85 billion people were reported to be at risk of *P. vivax* transmission in 2009 and Central and South East Asia contributed to 91% of it. Recent literature survey suggests that many complicated *P. vivax* malaria cases have been reported from Thailand, Brazil, Indonesia, Papua New Guinea, and India. In a review by Naing et al there was a description of eight studies (n = 27490) which reported comparable occurrence of severe malaria in both *P. vivax* and *P. falciparum*. Six studies were reported where the incidence of severe anemia was seen in both *P. vivax* infection and *P. falciparum* infection.

Dr Subash Varma, Director, PGIMER, Chandigarh, and Professor and Head, Department of Internal Medicine, presented a talk on malaria control strategies in the changing face of clinical features of *P. vivax* malaria.

Earlier complicated malaria cases were only observed in case of *P. falciparum* infection. But now the scenario is changing, i.e., high number of severe cases of malaria are also reported from *P. vivax* infection. Major complications observed during malarial infection include thrombocytopenia, leukopenia, acute respiratory distress syndrome, high bilirubin, acute renal failure, anemia, mucosal bleeding, cerebral malaria, hypotension, metabolic acidosis, and death. The exact cause of this change in the clinical symptoms of malaria due to *P. vivax* infection is still not clear. This may be because of variations in the parasitic genome or vector genome and modifications in the vector biting habits or drug resistance. Even patients with organ failure are reported from *P. vivax* malaria.

It is of utmost priority that further research is required to know why there is a change in the virulence, and whether any genetic mutation in *P. vivax* is responsible for this changing pattern.

### Chalcones in Malaria Research

Chalcones have very low molecular weight, it is easy and economic to prepare. These are stable nontoxic compounds. These features pulled scientists to check for their antimalarial activity to be used as an antimalarial drug.

Survey of literature reveals that various research groups have explored the potential of chalcones against *P. falciparum*. Yadav et al studied the efficiency of chalcones to act as antiplasmodial agent. They carried...
out in vitro studies. The research group synthesized 27 novel chalcone derivatives to test its antimalarial activity against asexual blood stages of P. falciparum [half maximal inhibitory concentration (IC50) antiplasmodial activity in vitro]. The most active compound was found to be 1-(4-benzimidazol-1-yl-phenyl)-3-(2,4-dimethoxyphenyl)-propan-1-one which showed IC50 of 1.1 lg/mL, while IC50 of the natural phytochemical, licochalcone A, is 1.43 lg/mL.

Bhattacharya et al26 also studied the effectiveness of chalcones to show antimalarial activity. They used chalcones with artemisinin. Synergistic effect was seen in vitro with this combination. The study revealed that chalcones when used with artemisinin reduce the formation of hemozoin in infected RBCs. The study therefore opens the door to novel artemisinin-based combination therapies. Go et al27 also studied the action of chalcones on infected RBCs. They reported that chalcones hinder the new penetration pathways to host RBC membrane, which are induced by the Plasmodium parasite. Different chalcones employ different pathways to show their antimalarial action. Sorbitol-induced pathway is one such permeation pathways to cause hemolysis of infected RBCs. Some chalcones inhibit this pathway and act as very active antimalarial agents.

In the oral presentations of MICROCON 2016, Ms Shweta Sinha from Postgraduate Institute of Medical Education & Research, Chandigarh presented work on evaluation of antimalarial activity of novel chalcone derivatives in Plasmodium berghei-infected murine model. She screened a series of new chalcone derivatives against P. falciparum strain under in vitro conditions. Out of all, three derivatives with most effective activity (A41, AV21, and AV27) were chosen for evaluation of its antimalarial activity in Plasmodium berghei strain infected with rodent Plasmodium strain. In terms of parasitemia A41, AV21, and AV27 showed highly significant result at the fifth day of treatment as compared with nontreated group (p < 0.001). Histopathological sections of liver and spleen also revealed the accumulation of more malaria pigment in nontreated group as compared with treated group. At the end it was suggested that these chalcone derivatives could be interesting antimalarials, but further studies are required for assessing the safety profile of these derivatives.

Malaria and Typhoid Coinfection

Ms Monika Matlani from Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, presented work on malaria and typhoid coinfection among febrile patients in a tertiary care center in New Delhi. She determined the prevalence of typhoid and malaria coinfection. Coinfection rate by using Widal test and RDT was found to be low, i.e., 3.4% (105/3010), as compared with other studies. However, gold standard tests for both the infections showed that true coinfection was present only in 1.6% cases.

In this regard, in a review entitled “Coinfection of typhoid and malaria” by Pradhan,28 true coinfection has been explained. In malaria, there is huge destruction of RBCs, which causes anemia in the patient. Red blood cell destruction leads to the accumulation of free iron in the liver. This availability of free iron in excess is harmful to the host defenses as it makes host more susceptible to intracellular pathogens like Salmonella typhimurium.29-31 Salmonella typhimurium needs iron for intracellular growth and survival.30-32 Iron in excess also leads to the weakening of phagocyte function, thereby supporting the bacterial growth further. So it is clear that anemic patients are more susceptible to typhoid infection and that is why there are many cases of malaria and typhoid coinfection.33,34

Prevalence of Malaria with Seasonal Variation

Ms Chaya AK from L.T.M. Medical College, Mumbai presented work on 3-year trend analysis of prevalence of malaria with seasonal variation in association with Plasmodium species. Plasmodium vivax (5.04–5.55%) was found to be more prevalent throughout the year than P. falciparum (2.04–3.74%) except in monsoon season when mixed infection was 3.52%. Prevalence of malaria was found to be more in adults (6.69–8.32%) than in children (0.66–1.27%). It was concluded that sustained antimalarial control measures should be intensified in monsoons.

Singh et al35 conducted similar prevalence study of malaria in a tertiary care hospital in Navi Mumbai. Patients attending the hospital, between January and December 2013, were enrolled for the study. Out of 4,878 suspected malaria cases, 809 (16.58%) were found to be positive for malaria. It was recorded that 54.76% were P. vivax cases, 17.80% were P. falciparum cases, and 27.44% were cases of mixed species. The study revealed that the prevalence of malarial infection showed seasonal variation. Most of the cases were reported during the time period of July to November. Maximum numbers of cases were reported in October. Prevalence of malaria was found to be twice higher in male patients than in female patients. Studies revealed that maximum number of cases were from the age 21 to 30 years.

Madhavan et al36 have also studied the prevalence of malaria with seasonal variation. They enrolled patients attending MGIMS, Sevagram, Wardha (Vidarbha region in Maharashtra). This study was carried out for 3 years. It was found that maximum malaria cases were reported
during September to November of every year. This clearly shows that increase in mosquito breeding after rainy season is responsible for the rise in the malarial cases.

**CONCLUSION**

In conclusion, the presentations as well as research in MICROCON was focused on the development of newer diagnostic techniques to detect malaria at a very low parasitic load, early diagnosis, drug resistance in malaria, and mechanism of drug resistance and combating drug resistance. The future research on malaria should focus on these areas.

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