Molecular Tools for Malaria Diagnosis: A Step towards Elimination

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Malaria entails a significant burden for many developing countries in the world. As per the WHO report in 2015, 91 countries had active malaria transmission and approximately 212 million people were affected with malaria disease worldwide [1]. Five species within the genus *Plasmodium* namely *P. falciparum* (*Pf*), *P. vivax* (*Pv*), *P. malariae* (*Pm*), *P. ovale* (*Po*) and *P. knowlesi* (*Pk*) are known to cause malaria disease in humans. The prevalence of *Pf* and *Pv* is higher in comparison to *Pm*, *Po* and *Pk* [2,3]. *Pf* infection has been known to be life threatening and responsible for most of malaria-related deaths worldwide. However, severe malaria cases due to *Pv* infection have also been reported [4,5]. This situation gets worse when the current diagnostic tools fail to detect malaria, especially at submicroscopic levels [6,7]. The success of control programs against the deadly malaria disease highly depends on rapid diagnosis and effective treatment. Traditionally, light microscopy method for the diagnosis of malaria has been considered as the gold standard, since it is cost-effective, quantitative, allows species identification and does not require advanced technology. However, low parasitemia and mixed infection could lead to misdiagnosis [8,9]. The use of rapid diagnostic tests (RDTs) is very common in malaria case management and elimination programs, particularly in remote areas where facilities for microscopy are not available [10]. Strikingly, the emergence and spread of deletions in the *pfhrp2* gene [key target antigen to detect *Pf* in commercially available RDTs] could adversely impact the life of an affected individual as a consequence of false negative diagnosis resulting in delayed or no treatment [11]. The molecular diagnosis of malaria parasites has a promising potential to be highly accurate and sensitive for detecting multiple species in afebrile individuals (low parasite counts but no symptoms) [12], who seem to sustain the transmission of the disease. However, the suitability of treating afebrile cases may be debatable [13,14]. The majority of molecular diagnostic assays still depend on 18S rRNA gene [15,16].

The new molecular methods which rely on repetitive genome targets could detect ≥0.03 parasites/μl blood [17]. Demas., *et al.* (2011) demonstrated the presence of 14 - 41 copies of Pvr47 and Pfr364 sequences corresponding to *Pv* and *Pf* parasites, respectively and reported their utility in malaria detection [18]. Similarly, Lucchi., *et al.* (2012) identified 7 copies of Pkr140 sequence in *Pk* genome [19]. Hofmann., *et al.* (2015) demonstrated the existence of 250 and 59 copies of telomere associated repetitive element 2 and *var* gene acidic terminal sequence respectively in *Pf* genome and developed an ultra-sensitive assay to detect *Pf* parasites using real-time PCR [17]. More recently, Gupta., *et al.* (2016) demonstrated the presence of PMLS152 and PvMLS110 sequences up to 44 and 34 times in the genomes of *Pf* and *Pv*, respectively [12]. The utility of multi-copy DNA sequences present throughout the malaria parasites genomes as a target for amplification, could further enhance the sensitivity of molecular assays for the identification of different *Plasmodium* species [12,17-19].

In a nutshell, the precise detection of afebrile malaria cases using molecular tools could significantly boost the success of malaria control programs. Therefore, molecular characterization of the *Plasmodium* species is highly recommended.

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