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REVIEW ARTICLE

Drug Metabolite as a Novel Tool for Measuring Antimalarial Drug Adherence

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Abstract: Malaria has been a major public health problem worldwide. The burden of malaria has been reduced by the adoption of *Artemisinin-Combination Therapy* (ACT) followed by primaquine dosage in malaria-endemic countries. However, evidences of non-adherence behavior lead to the discovery of antimalarial drug adherence to ensure a successful and satisfactory treatment of ACT, since it is the only available antimalarial drugs against asexual form of the parasite. Unstandardized questionnaires and limited effective alternative approaches have been the major obstacles to measure adherence. With rapid development of pharmacokinetic research, public health researchers can adopt the approach to measure adherence. Notwithstanding, the current structured questionnaire has explained in detail that the measurement and classification of adherence have produced satisfactory results. However, it is subject to social desirability bias. Therefore, in this review, we offer a new strategy combining structured questionnaire and drug metabolite as a novel consensus which eliminates biases. A new classification of adherence and graphical representation of practical strategy and other important factors are provided in this review. Thus, it initiates further works to conduct an intervention program to increase adherence level. Additionally, adherence behavior prevents the development of drug resistance and its spread, increases satisfactory cure rate and inhibits transmission by eliminating gametocyte inside host's body.

Keywords: Drug metabolite, *Artemisinin Combination Therapy*, Adherence, Novel approach, Measurement, Malaria.

1. INTRODUCTION

In 2015, the number of malaria cases worldwide reached 212 million (range 148-304 million). There is 14% decrease of malaria incidence worldwide from 2010 to 2015 with an approximate number of 245.000 cases to 212.000 cases, respectively. Most of the malaria cases occurred in African regions, followed by East Asia and Mediterranean. Indonesia has been reported to contribute malaria cases with an approximate percentage of 7% to all malaria cases in the world. WHO has documented in African region that malaria cases proportion supported with parasitological test escalated from 40% suspected cases in 2010 to 76% cases in 2015. This escalation is primarily caused by the enhancement of Rapid Diagnostic Test (RDT) utilization, which contributed 74% from diagnostic test within suspected cases in 2015 [1].

Since, ACT is known as an effective prompt treatment and prevention as well as intermittent mass drug administration strategy. The deployment of ACT has been widely adopted. The adoption of ACT increases every year. Eighty four countries have legally accepted ACT as their first line treatment against malaria; 60 of those countries supplied complimentary ACT in the public sector and 8 countries even had trials of subsidized ACT in the private sector [2 - 4].

The resistance problem of parasites to antimalarial drug has become a major public health concern and routinely changed drug regime over time. The failures of some drugs for treating malaria have been reported. However, some drugs are still investigated. Quinine as the oldest drug from quinoline group has been reported to develop resistance in

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1910 [5]. Parasites resistant to amodiaquine have been reported to appear in South America, Asia and East Africa [6 - 8] also the emergence of piperaquine-resistant parasites [9]. Treatment failure to mefloquine has occurred in Thailand in early 1990s [10, 11]. On the other hand, resistance to primaquine is a difficult entity to be quantified separately, because primaquine is used with blood schizontocidal agent [12, 13]. Furthermore, there is no report that has been convincingly demonstrated to lumefrantine. The complexity of antimalarial resistance deteriorates since the main treatment of artemisinin directed against resistant parasites has begun to appear [14 - 17]. Currently, nonsynonymous polymorphism at codon Y439H, R539T, I543T and C580Y observed in the kelch repeated region of K13 propller domain have been discovered to have higher resistance to artemisinin [18]. Another marker that is beneficial to be discovered, since Artemisinin-based Combination Therapy (ACT) is widely applied likewise the combination of dihydroartemisinin-piperaquine, has led to current findings of piperaquine resistance marker, which is *Plasmepsin 2-3* copy number. Patients with multicopy-*plasmepsin 2* parasites were 20 times more likely to experience treatment failure. Between 2002 and 2015, the proportion of parasites with multicopy *plasmepsin 2* increased steadily after the introduction of dihydroartemisinin–piperaquine. It also elevated the piperaquine 50% Inhibitory Concentrations (IC_{50}) of field clinical isolates of *Plasmodium falciparum*, hence strengthening the fact of its importance in surveillance application [19 - 21].

By these facts, it is imperative to monitor ACT treatment regimen. One of the approaches to monitoring the successful treatment regimen is by strictly inspecting adherence in a population. However, with no convincing results of previous studies and unstandardized methods, it has been poorly discovered. Therefore, the aim of this review is to provide a scientific basis of a novel strategy to measure adherence in an effective and precise way.

2. SITUATION OF COMPLIANCE AND ADHERENCE OF TAKING ACT MEDICATION IN POPULATION

Previous systematic reviews have described how antimalarial drugs were used among malaria infected patients [22]. From these reviews, patients' adherence to ACT varied from 78% adherence of a three-day course of AS+SP in Zambia [23] to maximum of 93% for AL in Uganda [24]. On other hand, adherence level of ACT in Kenya is <30% [25], in contrast to 100% of AL adherence in Malawi [26]. Adherence was discovered to be generally preferable when "interventions focusing on provider knowledge and behavior, packaging and provision of correct dosage" were performed [22]. A poor homogeneity between studies and large range of adherence level can be directed to the variability of the study settings, study design and ACT formulation, as well as studies measurement tools (questionnaire or interview), blinding protocol and the features of study design (RCT *vs* observational). Additionally, it would be imperative to consider the standardized definition of adherence, importantly a definition which integrates and considers duration, timing and frequency of dose. Without any standardized definition of adherence, the studies will not be comparable.

Findings of published factors were inconsistently described. Demographic background (such as sex, socio-economic status and age) does not seem to be the factor of adherence to ACT [27 - 39]. However, the age factor seems to be underpowered by some studies as other findings found that age was a risk factor of adherence. Younger people are more likely to be not adherent. Children less than five years were more likely not to be adherent to take ACT treatment in Malawi [28], while in Kenya children of 15 years old of age and more were less likely to adhere compared to older patients [27]. Although age is one of the associated risk factors of poor adherence to non-ACT regimen [40], formulation of ACT and communication campaigns should take into account age related factors. Another associated risk factor of ACT medication is vomiting. However, vomiting is a difficult entity to measure. It is negatively correlated with AQ+AS and AL [37, 39], although it is considered an exclusion criterion in some studies. As vomiting could be affected by treatment regimen and disease severity, the association of vomiting to non-adherent behavior should be done.

Generally, the study design for indentifying adherence level in ACT treatment is prospective observational or Randomized Controlled Trial (RCT). Some issues have appeared in relation to the study design. The retrospective observational study is lack of accuracy as sampled persons mostly recruited several weeks after treatment. Similarly, in cross-sectional design, recall bias is more likely to appear, thus under- or over-estimating adherence level. The adherence level is also commonly obtained from Malaria Indicator Survey (MIS), although the indication of adherence could be obtained notwithstanding such research does not include a suitable measurement. In addition, patients' understanding or familiarization of the drugs may not be adequate through this type of survey. The nomenclature of local terms of the drugs also needs to be considered for ensuring a correct understanding for local inhabitants. Prospective observational studies that interviewed patients or caregivers a day after the last day of drug prescription should possess preferable recall. Notwithstanding, the precision of instrument depends on the recruitment method of

patients/caregivers and their awareness or realization of upcoming follow up [36, 37, 40]. Randomized-Controlled-Trial (RCT) and pre-post designs also face similar problems, as the enrolment of patients requires informed consent of participation prior to taking part in the study. In most of previous studies, patients/caregivers were aware of their voluntarily participation and they may have changed their behaviour or attitude to be more adherent (*i.e.* Hawthorn effect).

Plenty studies have been conducted to discover adherence to anti-malarials for over a decade. However, their methodologies still lack standardization. An imprecise definition of adherence (probably adherent, probably non-adherent and non-adherent), which has been used for most of the studies, can result in misclassification of individuals' adherence, leading to either overestimation or underestimation of adherent behavior. These methods were used to assess the adherent behavior of non-ACT drugs [22]. Yet, they are still broadly utilized to assess adherence to ACT today.

Some studies used self-report as it is less expensive and easy to implement. However, despite the convenience, it still contains social desirability bias, which may be an overestimation of adherence. Currently, measurement tools of ACT adherence using questionnaire are unstandardized and the complexity is similar to household survey structure. For instance, a standardized questionnaire for neither HIV nor TB treatment regimens has been utilized to assess adherence of the treatment, with few of these tools are lengthy and detailed while the others are concise. It is still considered to be hypothetically proper adherence measurement (*e.g.* the Brief Medical Questionnaire (BMQ) and Morisky scale) [41 - 43]. On other hand, MEMS (Medical Event Monitory Services) and biological assays appear to be more objective and promising for measuring drug adherent behavior and may offer more precise adherence measurements. However, further scientific agreement is urgently demanded with regard to translation of bioassay data into a measurable entity for adherence/non-adherence. Although, the interpretation of this method is objective, measuring drug metabolites in the blood may be problematic [24, 30, 31, 44 - 46].

3. POPULATION PHARMACOKINETICS OF PIPERAQUINE AND PRIMAQUINE

3.1. Piperaquine

A number of antimalarials have been introduced and known to be resistant. All families of antimalarials have their pharmacokinetic properties which are unique and may partly explain their failure in treating malaria infected patients. Artemisinin combination therapy is suggested due to avoiding resistance by combining two different properties of pharmacokinetic of the drugs to expand effectiveness and efficacy. Mainly, the most highlighted property is the half-life of the drug. Since, artemisinin has short half-life and is concentration dependent, this drug needs additional long half-life partner drug which is time dependent in order to achieve greater clinical significance and avoid unexposed parasite to remain in the patients' body after artemisinin treatment. Other important pharmacokinetic properties of a drug are maximum concentration (Cmax), time at which maximum concentration is achieved (Tmax), and area under curve which is a function of time and concentration (AUC). These are denoted as secondary results of pharmacokinetic studies. The primary and secondary definitions of pharmacokinetic parameter are described in Table **1**.

Table 1. Description of pharmacokinetics parameter.

No	Abbreviated Parameter (unit measurement)	Definition
PRIMARY OUTCOMES		
1	CL/F (l/h)	It is the apparent elimination clearance
2	Vc/F (l)	It is the apparent volume of distribution of the central compartement
3	Q1/F (l/h)	These are the inter-compartment clearances between the central and the peripheral compartment
4	Q2/F (l/h)	
5	VP1/F (l)	These are the apparent volumes of distribution of the peripheral compartments
6	VP2/F (l)	
7	MTT (h)	It is the mean transit time of the absorption
8	Number of transit compartments	It is the number of transit compartments used in the absorption model
9	F	It is the relative bioavailability
10	RUV	It is the variance of the unexplained residual variability
COVARIATE RELATIONSHIP		
11	Scale	It is the difference between venous and capillary predictions
12	MF50 (years)	It is the maturation age (years) to reach 50% of the full elimination clearance

(Table 1) contd.....

No	Abbreviated Parameter (unit measurement)	Definition
13	$Hill_{MF}$	It is the hill function in the maturation equation, with an upper limit of 10
14	$Dose_F$	It represents the increase in relative bioavailability between dosing occasion
	SECONDARY OUTCOMES	
15	Cmax (ng/ml)	It is the maximum concentration
16	Tmax (h)	It is the time after dose to reach the maximum concentration
17	$T_{1/2}$ (d)	It is the terminal elimination half life
18	AUC∞ (h x ng/ml)	It is the area under the concentration-time curve from time 0 to infinity
19	Day 7 concentration	It is the venous plasma concentration at day 7 after dosing
	ADDITIONAL INFORMATION	
20	Coefficient of variation for inter-individual variability (IIV)	These are calculated as 100 x $(e^{variance} -1)^{1/2}$
21	Inter-occasion variability (IOV)	

A meta-analysis of a group of researchers aimed to identify and enhance piperaquine dosing from a pooled analysis has revealed several important points [47]. A three compartment disposition model with a transit compartment absorption model was discovered to precisely depict pooled meta-analysis data as a final model of piperaquine pharmacokinetics. The disease effect on pharmacokinetic would exist only during the early assessments (day 1-3) due to patients' body recovery. Furthermore, a disease could cause an alteration/effect on relative bioavailability, leading to an increase of piperaquine exposure in healthy people compared to infected patients. A 24% increase in relative bioavailability is noticed between dosing scheme, while the total daily milligram/kilogram dosage does not influence absorption. Secondary results indicate that Cmax (ng/ml) is estimated to be 248 (24.3-1070 (minimum-maximum), Tmax (hour) is 3.49 (1.13-10), $T_{1/2}$ (day) is 22.5 (9.15-52.3), AUC∞ (h*ng/ml) is 28.800 (2650-116.000) and day 7 concentration (ng/ml) is 28.1 (2.35-115).

3.2. Primaquine

Primaquine is known both as radical cure for hypnozoite form of *Plasmodium vivax* and gametocytocidal effect to *Plasmodium falciparum*. Thus, it prevents the relapse of *Plasmodium vivax* and disease transmission of *Plasmodium falciparum*. Therefore, it is imperative to understand the pharmacokinetic properties of primaquine.

Basically, after primaquine dosing, there are two important metabolites of primaquine in patients' body, primaquine and carboxy-primaquine. Primaquine is rapidly absorbed, attaining peak plasma concentration (median and range) of 167 (113-532) μg l^{-1} in 2 (1-4) hours. Afterwards, it decreases rapidly with an apparent terminal half-life of 6.1 (1.7-16.1) hours. There is no effect of partner drug on the values of any pharmacokinetics parameters of primaquine. On the other hand, the carboxylic acid metabolite of primaquine attains maximum concentrations (median and range) of 890 (553-3634) μg l^{-1} at 6 (3-16) hours. Afterwards, it declines to 346 (99-918) μg l^{-1} at 24 hours. Similar to primaquine, partner drug also has no effect on carboxy-primaquine. However, acute malaria does has a significant effect on the reduction of oral clearance of primaquine from 21.3 (15.9-73, 0) to 19.4 (9.3-24.7) hours. The area under curve for the carboxylic acid metabolite of primaquine is significantly greater following the administration of primaquine alone relative to the combination of quinine and primaquine [48]. Additionally, the primaquine pharmacokinetics data suggest that women have increased exposure to primaquine, which may put them at increased risk for toxicity when administered the same maintenance as men [49]. However, a short-higher dose of primaquine regimen is safe and well tolerated, which could improve primaquine compliance and effectiveness [50].

4. A NEW STRATEGY TO MEASURE ADHERENCE USING STRUCTURED QUESTIONNAIRE AND DRUG METABOLITES

As mentioned previously, due to the limitations of using questionnaire subjected to the community bias, further consensus is urgently needed. In 2015, Shiddique *et al.* [51] have established a new strategy to measure adherence while reducing previous practical and theoretical bias. The strategy included a direct observation of blister packaging of the drug (present and not-present) as well as a correct explanation of drugs that have been consumed by patients. A combination of the two indicators will be translated to adherence scale (certainly adhere, probably adhere, probably not adhere and certainly not adhere).

Although, satisfactory results have been obtained in the study [51], it is still leaving commentary, inevitable bias. Recall is the ultimate source of bias from these studies. Although, exclusion strategy may be undertaken, it leads to an increase of sample size which may not be feasible. Skepticism has arisen since it follows a complex structured questionnaire leaving broad criteria of adherence. In addition, no blister seen contributed 51% to the total consensus, indicating the lack of feasibility and may contribute significantly to methodological and statistical flaws. Further, with the existence of probable adherent or probable non-adherent which significantly contributed to the main finding previously indicates that it is not precise enough and hard to explain.

To overcome the drawbacks of the previous study, a more precise measurement will be advantageous. Drug metabolite is hypothetically the most precise measurement to assess adherence. However, challenge in ascertaining consensus of adherence is problematic for drug metabolite data. The ultimate matter of establishing the consensus is ascertaining the threshold. Thus, pharmacokinetic approaches can be adopted to understand in detail the dynamics of drug metabolism in the human body and what factors would contribute to the change of the drug naturally in the body.

Several variables of pharmacokinetic research can be used to generally understand factors that may affect the drug concentration in the body. These include relative bioavailability, effect of partner drug, Cmax, Tmax, T-half life, and day-7 concentration that already mentioned in the previous section. All the previously measured variables confirm the feasibility of measuring drug metabolite without any interference of neither human body nor the drug itself.

The terminal half-life of piperaquine, which is 63 days, indicates that it is still at a measurable amount until day 63. Table **2** shows a concentration of piperaquine at day 60 from several pharmacokinetic studies. Fig. (**1**) represents a declining trend of piperaquine metabolite from day 1 to any indicated termination time of each study. Generally, upper-middle quartile of the piperaquine metabolite at day 60 is more than 5 ng/ml, but when adjusted to lowest quartile, the concentration laid above 1 ng/ml. In contrast, 1-day course of drug prescription results in below 1 ng/ml concentration of the drug. This potential threshold of 1 ng/ml can be used to differentiate adherent from non-adherent individual in a population.

Table 2. Concentration of piperaquine at day 60.

No	Reference	Sites	Study population	Dose regimen	Malaria infection	Conc. Day 60 (approximate minimum-maximum)
1	[47]	Pooled analysis	Pooled analysis	Pooled analysis	Pf & healthy volunteers	>1 ng/ml (±1.6-20 ng/ml)
2	[52]	-	adults	Single dose	Healthy	<1 ng/ml (±0.3-0.9 ng/ml)
3	[52]	-	adults	3 days dose	Healthy	>1 ng/ml (±4-7 ng/ml)
4	[53]	Thailand	adults	3 days dose	Pf	>1 ng/ml (±1.15-17 ng/ml)
5	[54]	Thailand	Children and adults	3 days dose	Pf & Pv	Pf: 1.2 ng/ml Pv: 1.3 ng/ml
6	[55]	Vietnam	adults	3 days dose	Healthy	6 ng/ml

*Pf = *Plasmodium falciparum*
Pv = *Plasmodium vivax*

On other hand, although the utilization of primaquine is still restricted due to some reasons, it is the key player for malaria elimination by preventing transmission. Thus, measuring adherence to primaquine is crucial and impactful. The consensus of adherence measurement of primaquine will be completely different from piperaquine since it only has 1-day course of treatment. Thus, discriminating the number of days over which the patients consume the drugs does not need to be done. Rather, it only requires a measurement of blood metabolite in the form of absence and existence. Table **3** shows a summary of previously published works measuring the drug concentration. Based on terminal half-life of primaquine, which is 4 days, and Table **3** which shows the drug concentration at day-4 which is 80 ng/ml, then it is possible to measure the drug metabolite on day 5, indicative of maximum day at which the drug is still at measurable amount.

Table 3. Concentration of primaquine at 15, 25 and 100 hours.

No	Authors	Site	Infection	Metabolite	Conc. 15 h	Conc. 25	Conc 100 h
1	[49]	Thailand	Healthy	1. PPQ 2. Carboxy-PPQ	1. ±35 μg l^{-1} 2. ±790 μg l^{-1}	1. – 2. ±510 μg l^{-1}	1. – 2. -
2	[48]	Thailand	Pf	1. PPQ 2. Carboxy-PPQ	1. ±89 μg l^{-1} 2. ±240 μg l^{-1}	1. – 2. -	1. - 2. -

(Table 3) contd.....

No	Authors	Site	Infection	Metabolite	Conc. 15 h	Conc. 25	Conc 100 h
3	[49]	Vietnam	Healthy	1. PPQ 2. Carboxy-PPQ	1. - 2. -	1. ±4 ng/ml 2. ±1000 ng/ml	1. – 2. ±80 ng/ml

*PPQ = Primaquine

Pf = *Plasmodium falciparum*

As discussed above, an urgent demand of further effective consensus in measuring adherence leads to our perspective to establish a new effective strategy. Although, several bias have been found in a structured questionnaire strategy, it is still an important part to be carried out for obtaining reasons of non-adherent behavior if any. Thus, with a sufficient notion of the utilization of drug metabolite, a combined strategy involving both structured questionnaire and drug metabolite measurement will be more precise. In the elimination phase, it is common to conduct active surveillance in a population. Then, after mass screening, the drugs should be deployed to the positively infected persons. Afterwards, at day 6 (including day 0) the structured questionnaire which can follow previously published work (or with minor modification following prospective study) [51] can be directed to the patients with initial finger prick to measure primaquine drug metabolite. At day-6, informed consent should also be given to the patients avoiding patients' awareness or recognition of the study as aforementioned section. Finger prick also should be taken at day 60 to measure piperaquine metabolite from the patients' blood. Finally, final analysis can be done to ensure adherence based on a new classification of Table **4**. Graphical representation of the process can be seen in (Fig. **2**).

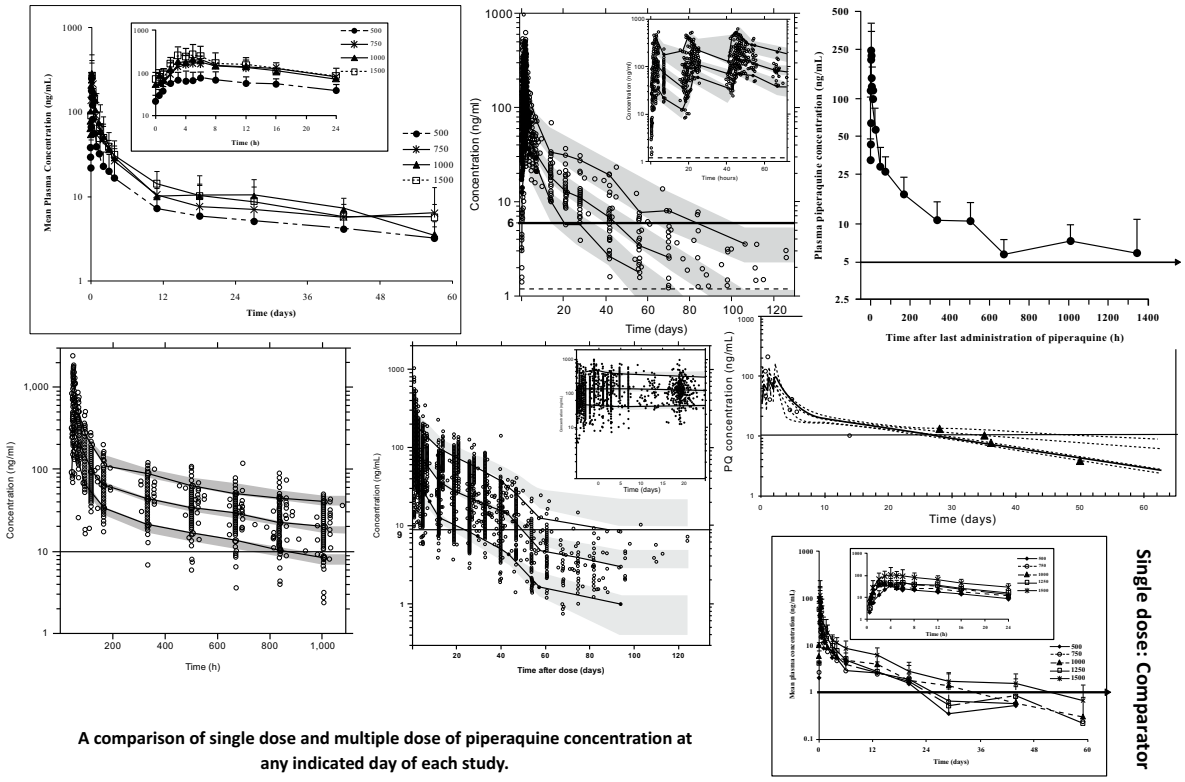

Fig. (1). Summary of picture from previously published pharmacokinetic studies for piperaquine. A graphic which is located on top-left-corner of the picture indicate a multiple dose concentration with 3-days course. A picture which is situated on bottom-right-corner exhibit a multiple dose concentration with 1-day course of the drug. All the 3-days course of the drug are in agreement of above-5 ng/ml concentration of the drug (the lower quartile is still above 1 ng/ml). While 1-day course regimen, with regards to the varying dosage of the drug, is below 1 ng/ml. It is indicative of potential threshold of measurable adherence assessment.

Table 4. Final consensus for a combination of structured questionnaire and drug metabolite measurement.

Structured Questionnaire [51]	Drug Metabolite	Final Classification
Certain non-adherence (pills remaining)	Drug concentration below threshold	Non-adherence (Certainty)
	Drug concentration above threshold	Adherence (elimination of recall bias)
Probable non-adherence (patient describes incomplete number of pills taken)	Drug concentration below threshold	Non-adherence (elimination of recall bias)
	Drug concentration above threshold	Adherence (elimination of recall bias)

(Table 4) contd.....

Structured Questionnaire [51]	Drug Metabolite	Final Classification
Probable non-adherence (patient describes incorrect time schedule or dosage)	Drug concentration below threshold	Non-adherence (elimination of recall bias)
	Drug concentration above threshold	Adherence (elimination of recall bias)
Probable adherence (patient describes correct number of pills taken, time schedule and dosage)	Drug concentration below threshold	Non-adherence (elimination of Hawthorn effect)
	Drug concentration above threshold	Adherence (Certainty)

Finally, Table **4** offers a new consensus of adherence which can be implemented in malaria-endemic countries. These new criteria satisfactorily establish a precise, adequate and preferable classification of adherence. Additionally, they eliminate all bias subjected to structured questionnaire. Interestingly, with an accurate classification based on drug metabolite enhanced by a structured questionnaire, the reasons underlying non-adherence behavior could also be assessed and become a significant input to public policies to improve the management and supervision of antimalarial drug treatment in a population.

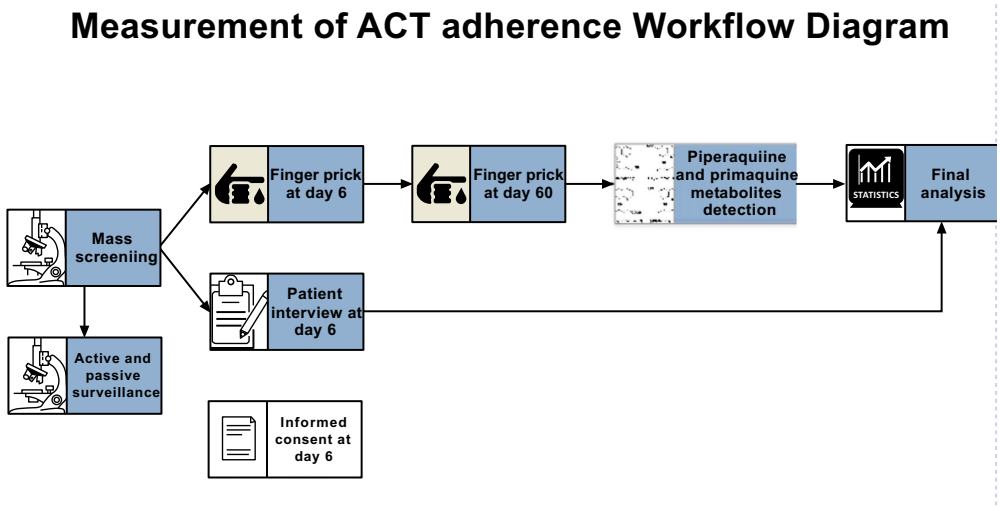

Fig. (2). A graphic representative of a new schematic strategy to measure antimalarial drug adherence in a population.

CONCLUSION

In the elimination phase of malaria, it is imperative to continuously monitor the program of malaria treatment and prevention. *Artemisinin-combination therapy* appears to be the only effective and available drug, while primaquine is the only drug against a hypnozoite form of *Plasmodium vivax* and gametocytocidal effect to *Plasmodium falciparum.* However, unstandardized questionnaire and unavailable alternative have made it difficult to overcome. Thus, in this review, we discuss and offer a new alternative and effective approach to measuring adherence in a population. A combination of structured questionnaire and drug metabolite with a novel schematic procedure and other graphical representations in this review offers a better and promising strategy to measure adherence in a population precisely and accurately, thus, initiating further works to conduct an intervention program to increase adherence level. Additionally, adherence behavior prevents the development of drug resistance and its spread, increases satisfactory cure rate and inhibits transmission by eliminating gametocyte inside the host's body.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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