

The effect of bed nets as malarial control on population dynamics of malaria vector

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ABSTRACT

*Malaria is a life threatening disease caused by parasites (*Plasmodium* spp.) which is transmitted through bite of infected mosquito. In 2012, about 219 million malaria cases and an estimated 660,000 deaths most of them are under five age children and pregnant women. Currently around three billion people (40% of the world population) are at risk of malaria. To eradicate the disease various control measures have been taken worldwide. From 1940s to 1960s DDT was used widely, and then replaced by other chemicals. The current intervention is mostly use of ITNs and/or IRS using pyrethroid insecticide. In various Africa countries such as Kenya, Gambia, PNG, etc proper and regular use of ITNs significantly reduced morbidity and mortality rate associated with malaria in under five age children and pregnant women. However, use of pyrethroid insecticide results in the development of resistance mosquito species throughout malaria epidemic regions. Resistance development mechanisms are many and complex including behavioural or physiological change, target site alteration and metabolic processes. However, target site as well as metabolic resistance is assumed to be the main types of resistance mechanisms. A recent research result indicated that the vectors show behavioural change to avoid a contact with insecticides.*

Key words; malaria, insecticide treated nets, indoor residual spray, physiological change, target site alteration.

INTRODUCTION

Malaria is a life threatening disease caused by *Plasmodium* spp parasites transmitted to people through bite of infected mosquito [1]. The parasites are spread to people through the bites of infected vector (*Anopheles* mosquito) which bites mainly between dusk and dawn. There are four parasite species that will cause malaria in human these are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale* [1]. In 2012 about 219 million cases of malaria and an estimated 660,000 deaths most of them are under five children and pregnant mothers. The distribution and transmission of the disease is limited between 640 North and 320 South latitude, below 2000m altitude and requires minimum temperature to complete its development into parasite [2]. Currently around three billion people, about 40% of the world population are at risk of malaria exposure [2]. To eliminate this devastating disease various control measures have been taken worldwide. For instance, DDT was used in the 1940s by many national malaria control program for indoor residual spraying (IRS), this program able to reduce the population at risk of malaria up to 50% [2]. The other control strategy is use of insecticide treated nets (ITNs). This method is effective against mosquito bites and reduced the morbidity and mortality rate associated with malarial disease [3, 4]. The use of ITNs as a malarial control policy in many countries starts in the mid of 1990s but the coverage is still lower [4]. Eventhough, different malaria vector control strategies (ITNs, IRS, and LLTN) are implemented still the distribution and transmission of the disease is very rapid and takes many lives every year.

The objective of this review paper is to give an insight on repeated use of insecticides and bed nets (ITNs) as a malaria control measures and its implications on the population dynamics of the malaria vectors.

Bed Nets as a Vector Protective Tool

Use of bed nets against a mosquito bites was proposed more than 70 years ago before the role of mosquito in the malaria disease transmission was recognized [5]. The use of ITNs come to mind of the public health experts 20 years back when they tried to evaluate the effect of pyrethroid insecticides on reduction of mosquito in Africa and Asia [4]. In 2008-2010 more than 254 millions of ITNs were distributed to malaria epidemic countries of Africa, which results in a significant reduction in morbidity and mortality rate associated to malaria disease [6]. A research report in Kenya indicates that ITNs have been distributed mainly to pregnant women and children under five and use of ITNs was increased from 7% in 2004 to 67% in 2006, and hence significantly reduced the malaria death by 44 % [7, 8]. In Kenya ITNs user children show a significant reduction in mortality rate (11.3 per-1000 person-year) than non user of ITNs children of same age (17.9 per-1000 person-year) [8]. Result in Gambia also indicated that children sleep under bed net got fewer malaria cases [5]. Similarly in Papau New Guinea (PNG) use of bed net significantly protect against *Plasmodium falciparum*. Another research report indicated that Japanese soldier stationed in Taiwan one battalion sleep with bed nets and other battalion without, 259 malaria cases were observed in those sleep without bed nets [5]. In India two malaria endemic districts (Malkangiril, and Koraput) to evaluate the benefits of bed net use, the result showed that the ITNs users were finding fewer mosquitoes inside their house at night and reduced malaria and undisturbed sleep at night [9]. The use of ITNs is one of the best ways to reduce the malaria burden in Africa. Proper and regular use of ITNs can reduce the mortality rate in children aged below five by 20 % [10]. In India (malaria endemic district) to evaluate the efficacy of the chemical (deltamethrin) as ITNs and IRS for malaria control the result indicated that higher reduction of malaria cases in ITNs than IRS. The incidence rate was 61.5% for control, 43.3% for spray and 28.1% per 1000-person-year [11]. In sub-Saharan Africa, Latin America, Thailand, Pakistan, and Iran a research report indicated that use of ITNs reduced malaria cases by 50% both *P.falciparum* and *P. vivax* infection [4].

Insecticide as Vector Control

Currently, use of ITNs, IRS, and spraying (fogging) are the common chemical control strategies to limit the vectors [5, 6]. The choice of control methods depends mostly on the behavior of the mosquito. For instance, for indoor, night biters such as *An.gambia* and *Culex quinquefasciatus* use of ITN and IRS are effective method.

Currently only four classes of insecticides have been approved by WHO for malaria control [6]. Starting from the 1940s DDT was widely used insecticide for the control and eradication of the vectors. Now a day's malaria vector control program depends mostly on the use IRS and / or ITNs. These control strategies contributed a lot in the reduction of the vector in Africa and other malaria endemic areas [3, 5, 6]. The current malaria vector control strategies based on pyrethroid insecticide (the only recommended chemical for ITNs use by WHO for the last 30 years) face a great problem due to the development of resistance by the vector throughout the malaria endemic countries. This is thought to be repeated use of the same chemical for more than three decades play key role for resistance development [3, 6, 7, 12, 13].

Insecticides Resistance Development

Many scholars give definition to insect resistance development to a give insecticide. According to [12] 'the developed ability in strain of insects to tolerate dose of insecticides which prove lethal to the majority of individuals in a normal population of the same species'. The ability of an insect to withstand the effects of an insecticide by becoming resistance to its effects by means of natural selection and mutations [7]. Development of resistance is dependent on the genetic variability of insect population and the mutation that occurs by selection pressure. The first resistance in mosquito to DDT was observed in Florida, USA in 1947 after four years use of it. Currently more than 500 species of arthropods, including 109 mosquito species found resistance to organochlorine, particularly to DDT and dieldrin [12]. A research report indicated the number of resistance mosquito species was 59 *anopheline* and 39 *culicine* [14]. Malaria vector resistance development mechanisms are many and complex, it includes behavioral or physiological change (avoidance, altered penetration, etc), sequestration, target site alteration, and bio-degradation (metabolic processes) [6]. It is assumed that there are two main types of resistance mechanisms; these are target site resistance and metabolic resistance. The target site resistance is well studied and understood; whereas the metabolic resistance is more complex but recently the main enzyme responsible for it is identified [7]. Physiological and behavioural change will also contribute for the development of resistance [6, 7, 13, 14]. In *anopheles* mosquito, high

level of glutathione-S-transferases causing DDT resistance, elevated level of P450 mono oxygenases along with altered sodium channel (kdr-knock down resistance) give resistance to pyrethroid, esterase enzymes is responsible for organophosphorous resistance in mosquito whereas, altered malathion carboxyl- estrases confer resistance to malathion [6,13,14,15].

Target Site Resistance

Target site resistance is a change in the target site that will reduce the binding of the insecticides and increase the metabolic rate and hence lower the insecticides that will bind to the target site [3]. Or a change in the amino acid which is responsible for insecticide binding that causes the insecticide less effective or totally ineffective. Acetylcholinesterase is targeted by Malathion, and carbamate insecticides, on the other hand, organochlorine (DDT) and synthetic pyrethroid acts on sodium channel [14]. pyrethroid, organochlorine, etc target the voltage gated sodium channel in the insect's neuron. It's binding delay closing of the channel and prolonging the action potential and will cause paralysis and death of insect. The resistance will develop due to the prolonged exposure to the insecticide. A mutation in sodium ion channel leads to resistance to pyrethroid in a variety of insects. Target site resistance to pyrethroid and DDT in *An.gambiae* is mostly related to a single point mutation, it is commonly called knock down resistance (kdr) [3, 13]. The mutation caused a voltage gated sodium channel insensitive to pyrethroid and DDT. Currently two kdr mutations were identified in *An.gambiae*, West and East Africa. The West Africa kdr is known by leucine is replaced by phenylalanine at position 1014 of the voltage gated sodium channel, on other hand East Africa kdr is leucine is substituted by serine at the same position of the sodium channel [7, 13]. A recent research result in Kenya indicates that there is an association with a high distribution of ITNs and kdr. kdr is highly associated with *An. gambiae*, rare in *An. arabiensis* and not observed in *An. funestus*. On other hand, P450 or glutathion-S-transferases related pyrethroid resistance is frequent in *An. arabiensis* and *An. funestus* [7].

Metabolic Resistance

“Metabolic resistance is an over expression of enzymes capable of detoxifying or sequestering insecticides and /or amino acid substitution within these enzymes which alter the affinity of the enzymes for the insecticides” [3]. Metabolic resistance occurs when one or more enzymes of insect will be involved in the detoxification of the insecticide before it will bind to the target site [13, 14]. Metabolic resistance is principally based on the three enzyme families, such as cytochrome P450 monooxygenases (P450s), carboxylesterases, and glutathion-S-transferases. Each enzyme family contain various genes which will involve in insect metabolic process however, only few numbers of these genes will be directly involved in detoxification of the insecticides [4, 13]. For instance, cytochrome P450 enzyme family is responsible for detoxification of pyrethroid in insects, 111 P450 enzymes is effective particularly in *An. gambiae*. A micro-array based investigation shows that three candidates of P450 genes (CYP6P3, CYP6M2, and CYPZ2) were frequently overexpressed in pyrethroid resistance population of *An.gambiae*, however; only CYP6P3 & CYP6M2 can metabolize the pyrethroid chemical. Recent research report in *An. funestus* indicated that the putative ortholog of *An. gambiae* CPY6P3 and CYP6P9 have been identified, which will provide resistance against pyrethroid chemical in this species [4, 6]. Micro-array based study in *An. gambiae* showed that many P450s enzymes (CYP6M2, CYP6P3, CYP6Z1 and CYP6Z2, CYP325A3) are associated with pyrethroid resistance. Of these CYP6P3 and CYP6M2 are most widely overexpressed in the field population (6). In Africa pyrethroid resistance mosquito is wide spread. The first resistance case was reported in 1993 in Cote div ore. But now it is spreads through West, Central and East Africa [3]. In Ghana, DDT resistance population of *An.gambiae* show high level of CYP6M2 gene expression, on the other hand, CYP6Z1 gene is highly expressed in both Pyrethroid and DDT resistance strain. In *An. funestus*, CYP6P9, CYP6P4, CYP6Z1, CYP6Z3 and CYP6M7 genes expression are high related to pyrethroid resistance. Increased level of CYP6P9 is frequently observed in pyrethroid resistance both in laboratory and field population in Mozambique, Uganda, and Benin [6]. The resistance development pattern of mosquito depends on the species and the type of the chemicals used. For instance, in South Africa Kwazulu Nata district *An. funestus* were reduced by use of DDT but when pyrethroid spray the disease reappeared in an alarming rate, which mean that this species was resistance to pyrethroid not to DDT [3]. In benin *An. gambiae* s.s populations are highly resistant to pyrethroid but the *An. funestus* population is not resistance [16]. A recent study in Africa shows that there is an increase in the frequency of resistance alleles in *An. gambiae*, this might be due to the selection pressure on the malaria vector, partly scale up of ITNs and pyrethroid use in IRS [3, 7]. In many insects insensitive to acetylcholinesterase (AchE) leads to resistance to organophosphate and carbamate insecticides. Two mosquito species (*A.gambiae* and *culex pipiens*) ace gene (ace-1) is responsible for AchE insensitivity and resistance development [17]. A recent research report indicated that high insensitivity in *A.gambiae* and *c. pipiens* was due to the replacement of glycine amino acid by serine by single gene mutation in ace-1gene [17].

Behavioural Resistance

Behavioral resistance describes “any modification in insect behavior that helps to avoid the contact and /or lethal effects of the insecticides” [3]. So far there are many reports of mosquito changing their behaviors due to intensive use of insecticides (ITN and /or IRS) but there is no sufficient data to proof the change is genetic or adaptive response [3]. A new research result indicated that the malaria vector undergoes a behavioral change to avoid a contact with insecticides either by biting at outdoor and /or in the late evening [16]. The behavioral change will be by a selection pressure and /or phenotypic plasticity in response to wide coverage of Long Lasting Insecticide treated Nest (LLINs) and / or IRS. For instance, in Kenya there is a shift in malaria vector type, from *An. arabiensis* to *An. gambiae* following to an intensive use of LLIN [16]. A recent research done in Benin (Tokoli and Lokohoue district) shows that there is a clear behavioral shift of *An. funestus* in there biting times. According to the research result, in the first round of the research the peak biting time of the mosquito was between midnight and 01:00 in Tokoli district. In round two (year after use of LLINs), two peak biting times were recorded in the same district, between 00:00 - 01:00 and between 03:00- 04:00. This shows a shift in biting time between round one round two of the research. In round three (three years after the use of LLINs) one peak biting hour was recognized that was between 04:00-06:00 [16]. In general speaking from 2008-2011 the *An. funestus* mosquito shifts its peak biting time from 02:00 to 05:00 [16]. On the other district, Lokohoue, in the first round of the research, no peak biting time was scored. In this district before distribution of LLINs the peak biting times was around 03:00, but after the use of LLINs the time shifts to 04:00 and 05:00 in round two and three respectively [16]. On the other hand, concerning to the outdoor biting proportion of this mosquito in this two districts, in the first round, it was found 45.6% and 44.6%. In Tokoli district there was a dramatic shift in the proportion of outdoor biting in the second and third round of the research, 68.1% and 60.9% respectively. In contrast almost no shift in the outdoor biting behavior in the Lokohoue district in the second and third round of the research (44.2% and 46.7 respectively) [16]. There is also a report in Tanzania *An. funestus* shifts from indoor to outdoor biting following to wide coverage of pyrethroid impregnated nets [16]. This and other research report indicated that there is a behavioral change in the malaria mosquito in response to the ITNS and / or IRS.

DISCUSSION

Malaria is one of the major healths and development obstacle in Africa. It takes large number of very young children and pregnant women life every year [10]. The use of ITNs come to mind of the public health experts 20 years back when they tried to evaluate the effect of pyrethroid insecticides on reduction of mosquito in Africa and Asia [5]. Currently ITNs is one of the main malaria vector control intervention strategy used by many countries as a national malaria control mechanism. in Kenya , ITNs use by children aged less than five significantly increased from 7% in 2004 to 23.5% in the next year and to 67% in 2006 [7, 8]. Even though the number of INT users in Africa increased every year still the distribution and transmission of the disease is continued, why it so? Resistance of the vector to the pyrethroid (pyrethroid insecticides used 40% for IRS and 100% as ITNs by WHO) is the main challenge in many Africa countries such as Kenya, Ghana, Tanzania, Mozambique, Benin, etc [3, 7, 13, 16].The mechanisms of resistance development in malaria vector are many and complex, such as behavioral change, physiological resistance, metabolic based resistance, target site resistance, etc. However, the main resistance development mechanisms are metabolic based as well as target site resistance, currently behavioural resistance also get an attention [3, 6, 13, 15, 16].

In metabolic resistance when one or more enzymes of the insect will be involved in the detoxification of the insecticide before it will bind to the target site [13, 14]. Metabolic resistance is principally based on three enzyme families, such as cytochrome P450 monooxygenases (P450s), carboxylesterases, and glutathion-S-transferases , each enzymes will involved the detoxification of the insecticides [4, 13]. In target site resistance a change in the amino acid which is responsible for insecticide binding causes the insecticide action less effective or totally ineffective., acetylcholinesterase is targeted by organophosphorous (malathion) and carbamate (propoxur) insecticides, on the other hand, organochlorine (DDT) and synthetic pyrethroid acts on sodium channel [14]. In a very recent research work in Benin two districts shows that a malaria undergoes a behavioural change (a change in a biting period) in response to a wide coverage of LLIN, the research also indicated that in Tanzania the *An. funestus* bites more frequently in the outdoor than indoor in response to a wide distribution of pyrethroid treated bed nets [16]. It is obvious that high coverage of ITNs and/ or IRS cause a dynamic shift in the malaria vector population which will impose great problem in the control of the disease in Africa.

Prospective

From the 1940-1960s malaria vector control strategy was depends solely on the use of DDT and it was effective, but later replaced by other chemicals due to the resistance development by the vector. Currently, the vector control program is mostly relay on the use of pyrethroid chemical either ITNs and /or IRS form for many years. But there are many reports for the development of resistance by the vector to this insecticide. In order to avoid or reduce the resistance development pattern the current malaria control approach (intensive use of ITN and / or IRS) should be diversified and intergraded with other approaches. Control strategy should consider use of biological methods to reduce the disease distribution and limit the resistance development of the vector. Other methods, like use of genetically modified mosquitoes (male sterile) to reduce the reproduction rate and to lower the next generation mosquito population density. In recent report there is an indication of a behavioural change in the malarial vector in the their peak biting period and outdoor biting character this issue should be well studied because these changes are associated with wide coverage of ITNs and IRS and results in change in malaria vectors population.

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