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Bioassay evaluation on the efficacy of α-cypermethrin impregnated into long lasting insecticide treated nets using *Anopheles stephensi*

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ABSTRACT

Objective: To evaluate the bioefficacy of α-cypermethrin impregnated into long lasting insecticide treated nets (LLITNs- INTERCEPTOR®) against main malaria vector, *Anopheles stephensi (An. stephensi)*. **Methods:** The effectiveness of bed net impregnated with α-cypermethrin (INTERCEPTOR®) with washing was evaluated. The washing procedure and bioassay tests were carried out according to the WHO-recommended methods. Malaria vector, *An. stephensi* was exposed to impregnated bed net for three minutes and then mortality measured after 24 h recovery period. Knockdown was also measured according to the logarithmic times.
Results: Result of cone bioassay method showed that bioefficacy of α-cypermethrin decreased from 100% in unwashed to 15% in 20 washes. KT_{50} was measure as one minute in one wash and increased to 40 min in 20 washes. **Discussion:** Findings of this study provide guideline for malaria vector control authorities and people using pyrethroid-impregnated bed nets.

1. Introduction

Malaria is the most important vector-borne disease in world. It is estimated that 80% to 90% of the 300 million annual cases and one million deaths. The global strategy adopted by the World Health Organization (WHO) recommended an integrated management of the disease, including selective vector control[1]. Selective vector control is defined as: application of site-specific targeted use of different and cost-effective vector control methods alone or in combination to reduce human-vector contact. Insecticide-treated nets (ITNs) are one of the main vector control tools against malaria and recommended at different epidemiological strata of malaria. They are as effective as indoor residual spraying (IRS) and strongly advocated for malaria prevention. Various methods for mosquito control have been suggested. An important innovation touring the past decade is the international introduction of long lasting impregnated nets (LLINs) for preservation against malaria transmission. Pyrethroids are today the only group of insecticides advocated for the impregnation of mosquito nets due to their rapid knock-down effects and high insecticidal potency at low dosages combined with relative safety for human contact, domestic handling and their low mammalian toxicity. Recently , WHO recommended long-lasting insecticidal mosquito nets for malaria control, they are : Duranet® Alpha-cypermethrin incorporated into polyethylene; Interceptor® Alpha-cypermethrin coated on polyester; Netprotect®, deltamethrin incorporated into polyethylene; Olyset®, permethrin incorporated into polyethylene; PermaNet® 2.0, deltamethrin coated on polyester; PermaNet® 2.5, deltamethrin coated on polyester with strengthened border; PermaNet® 3.0, combination of deltamethrin coated on polyesterwith strengthened border (side panels) and deltamethrin and synergist (PBO) incorporated into polyethylene (roof)[2]. There is a vested interest for LLINs for malaria prevention. These products now represent more than 80% of nets currently ordered

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by national control programmes and institutional buyers purchase millions of these nets every month. Advantages of LLINs can be categorized as: ready to use, dirt-repellent net, long-lasting efficacy, and high durability to washing and wide mesh size to provide good airflow. Pyrethroid-impregnated nets have an impact on reducing mortality and morbidity due to malaria If the coverage is good they also provide community protection by significantly reducing the vector population.

Iran has been classified in to four different strata according to the epidemiology of disease[3]. A total of 6 222 malaria cases had been reported in Iran in the last year. The disease is a major health problem in south-east of Iran. It is unstable with two seasonal peaks mainly in spring and autumn. Outbreaks usually occur after rainy season. South-eastern of Iran includes the provinces of Sistan & Baluchistan, Hormozgan and the tropical areas of Kerman Province. Malaria remains a major public health problem in these areas where about 80% of all malaria cases in the country occur. In this part of the country six anopheline mosquitoes including *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles dthali*, *Anopheles fluviatilis*, *Anopheles superpictus*, and *Anopheles pulcherrimus* are known to be the malaria vectors[4-6]. *Anopheles sacharovi* and *Anopheles maculipennis* play role in northern part of the country[7,8]. According to the national strategic plan sponsored scheme supported by Global Fund, the LLINs are recommended for malaria control purposes, their performance should be monitored in the field under various ecological settings to assess their durability and long-term effectiveness for malaria prevention and control. Strategic plan of each country should be carefully designed to preserve the effectiveness of this method of control.

Interceptor® net is an α-cypermethrin long-lasting insecticidal (coated) mosquito net (LN) manufactured by BASF (Germany), with the target dose of 200 mg/m^2 of the polyester fabric. Applied objectives of current research were to examine the bioefficacy of this product against main malaria vector, *An. stephensi* for future use.

2. Materials and methods

2.1. Net purchasing

The net, α-cypermethrin, Interceptor® (LLINs) was provided by BASF chemical company, Ludwigshafen, Germany. This polyester mosquito net was 100 denier. Mosquito net were industrially treated with α-cypermethrin EW (200 mg a.i. /m^2) by the manufacturer. One piece (25 cm×25 cm) of net was cut. One piece was for cone bioassay. A net was left untreated (negative control). one piece from the same net (25 cm×25 cm) was used for cone bioassay before and after every washing(*e.g.* 0×, 1×, 2×, 3×, 6×, 8×, 15×, 20×), ensuring WHO standard washing procedure. The piece nets was washed and dried once a week.

2.2. Washing procedure

This procedure is recommended by WHO (2005)[9]. Net sample (25 cm×25 cm) was individually introduced into 1-liter beaker containing 0.5 liter deionized water, with 2 g/L soap "Le chat"(added and fully dissolved just before introduction of net sample). Beaker was immediately introduced into a water bath at 30 ℃ and shaken for 10 min at 155 rounds per minutes. The sample was then removed and rinsed twice for 10 min in clean, deionized water in the same shaking conditions as stated above. Net was dried at room temperature and stored at 30 ℃ in the dark between washes.

2.3. Detergent "Le chat" supply

The detergent was supplied by the Institut de Recherche pour le Development, Montpellier Cedex 1, France.

2.4. Mosquito rearing

For utilizing the cone bioassay for the determination of biological efficacy of α-cypermethrin on long-lasting impregnated net, the non-blood fed, 2-3 d old susceptible female *An. stephensi* (BEECH strain), susceptible to all pyrethroids reared in the insectary at the School of Public Health & Institute of Health Research, Tehran University of Medical Sciences. Then they were employed for all experiments.

2.5. Conical bioassay

1 to 3 d after treatment of the netting materials and 24 h after each washing, the netting sample were subjected to standard WHO bioassays. Mosquitoes were exposed to netting samples for 3 min after which they were held for 24 h with access to 10% sugar solution. Four cones gently fitted on the net. A net flap was made inside the cone to reduce the chance of mosquitoes resting on the cone instead of on the treated net. Five female mosquitoes introduced at a time in each cone with appropriate replicates per net sample. In cone bioassays, five mosquitoes were introduced into a cone at a time. 20 other batches of each mosquito were exposed to netting from untreated nets as control. Time interval between each set of a "4 cone" was as brief as possible. Mosquitoes from the first 4 cones, tested were grouped in one plastic cup (total of 20). Knock-down was measured in logarithmic interval times up to 64 min post-exposure and mortality after 24 h. At least 100 mosquitoes were tested on a netting sample (25 cm×25 cm)[9].

2.6. Probit regression line for calculation of KT_{50}

For plotting regression line, a computer programme was used[10].

3. Results

The mortality of *An. stephensi* exposed to INTERCEPTOR® nets by using conical test decreased from 100% in unwashed net to 15% after 20 washes. There was no significant difference in KT_{50} and 1 h knockdown between unwashed and 20 washes (Table1 & Figure1, 2). KD_{50}% time was diminished from 3.917 4 in unwashed net to 40.610 0 in 20 washes (Figure 3).

Table 1
Parameters of mortality and knockdown time of *An. stephensi* exposed to Interceptor® nets using conical test at laboratory condition.

No. of wash	A	B± SE	KT_{50} 95% CL	KT_{90}± 95% CL	χ^2 (heterogeneity)		Mortality(%) ±Error Bar	*P*-value
					Calculated	Table		
No wash	1.122 2	1.892 5± 0.276 0	3.917 4 (2.3979-5.927 3)	18.628 9(11.188 6-47.751 0)	19.297	16.7	100	<0.05
1	0.195 7	1.976 2± 0.261 0	0.796 1 (0.5284-1.043 2)	3.544 0(2.857 7-4.737 0)	0.815	16.7	100	>0.05
2	0.403 1	2.208 2± 0.218 0	1.522 5 (1.2314-1.809 8)	5.793 6(4.735 0-7.565 2)	1.858	16.7	100	>0.05
3	0.626 1	1.488 5± 0.252 0	2.634 2(1.5401-3.901 0)	25.455 0(20.121 8-34.162 7)	3.908	16.7	100	>0.05
6	1.343 4	2.395 7± 0.361 0	3.637 2(2.3861-5.295 8)	12.465 6(10.297 3-15.833 0)	20.397	16.7	97.5±1.0	<0.05
8	0.968 7	1.923 9± 0.281 0	3.188 1(1.9379-3.744 2)	14.780 5(9.090 2-36.076 6)	18.016	16.7	88.8±2.2	<0.05
15	0.807 7	1.837 5± 0.293 0	3.751 5(1.5185-4.250 6)	13.709 9(8.159 0-37.5718)	19.759	16.7	72.5±3.2	<0.05
20	1.382 9	0.859 7± 0.103 0	40.610 0(27.1697-72.158 5)	1257.292 4(473.681 1-5926.437 9)	7.673	16.7	15.0±2.5	>0.05

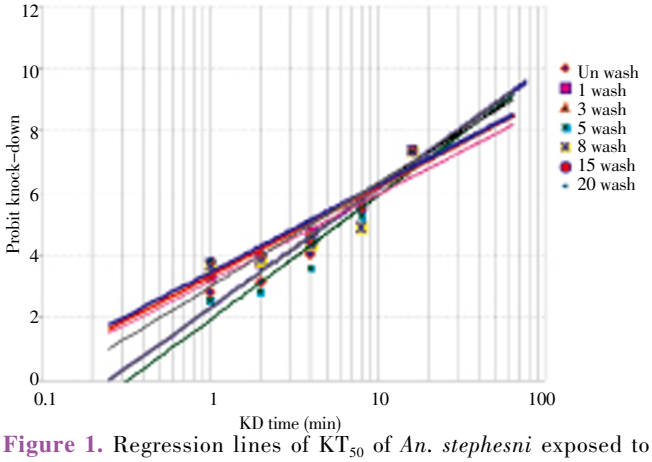

Figure 1. Regression lines of KT_{50} of *An. stephesni* exposed to INTERCEPTOR®

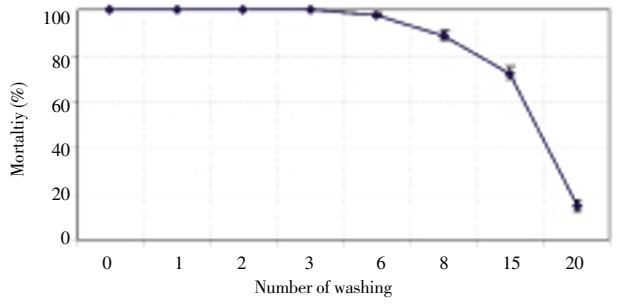

Figure 2. Mortality of *An. stephensi* exposed to different washes of INTERCEPTOR®

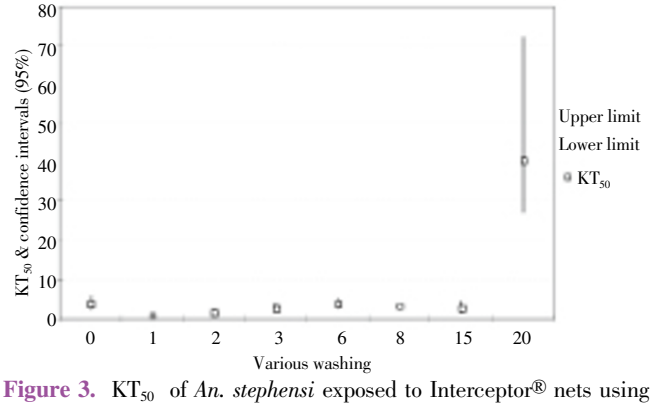

Figure 3. KT_{50} of *An. stephensi* exposed to Interceptor® nets using cone test at laboratory condition with various washing.

4. Discussion

There are several reports on different aspects of malaria vectors in the country for malaria vector control[11-13]. The present study determined the efficacy and wash-resistance of pyrethroid net named Interceptor® (LLINs impregnated with α-cypermethrin with the target dose of 200 mg/m^2) using cone bioassay method. Result of cone bioassay method on net revealed 100% mortality on unwashed net until 3 washes. In contrary, after three washes, the mortality was increased of 100% to 97.5% in cone test. KT_{50} on unwashed until 20× washed nets to one washed nets was 3.917 4 and 0.796 1 in cone test, respectively, that this divulged coated α-cypermethrin release of filaments nets after first washing and following that toxicity increased in bed net. Our result shows that around 1,2,3,6, × washings with "Le chat" soaps, the biological efficacy of Interceptor® against laboratory-bred mosquitoes were similar to unused and unwashed nets.

The wash resistance and efficacy of the Interceptor nets in the laboratory for two series (S-60 and S-61) of net samples provided by the manufacturer was determined. After 20, 25, and 30 standards washes (WHO washing procedure), samples were tested in bioassays, and using a fully susceptible *Anopheles gambiae* s.s. strain (Kisumu). For one sample of each series, KD effect after 20 washes was 100% but decreased below 95% after 25 washes. Mortality was below 80% for all assays. Both series met the WHO criteria at 20 washes (>80% mortality and/or >95% KD) [14].

The efficacy and wash resistance of Interceptor® according to the WHO guidelines for laboratory testing of LNs10 was evaluated. Two series (S-60 and S-61), each of eight samples (25 cm ×25 cm) were used for that purpose. For each series, two samples were tested without any wash, others were standard washed 5, 10 or 20 times by replicates of two. Bioassays were carried out 1, 2, 4 and 8 d after the end of the washing cycle. KD was observed after 3 min of exposure and 60 min of holding, and mortality after 24 h. Both series of nets showed good performances against susceptible *Anopheles gambiae* s.s. (Kisumu strain) in terms of efficacy and wash resistance. The KD was above the critical value of 95%, in one hour post-exposure, for all tests even after 20 washes. Mortality dropped down below the cut-off value of 80% at 20 washes.

They concluded that laboratory studies for two net series of the manufacturer demonstrated good and similar

performances against *Anopheles gambiae* in terms of efficacy and wash resistance. The Interceptor® nets met the WHOPES Phase I criteria of a KD effect above the 95% after 20 washes. Mortality dropped below the cut-off value of 80% after 20 washes. Maximum bioavailability was achieved with that [14]

Many fields research exhibited that if pyrethroid impregnated bednets was not exposed to washing, the residual effect of insecticide will be remained up to 6 to 12 months. This residual effect depends on type and quantities of insecticide used as well as type and characteristics of bed net. In practice, washing methods, type of detergents, the numbers and method of washing, are not as the same as in different parts of the world. It is determined that remaining soap on nets will demolish the molecular structure of pyrethroids. For instance, the number of washing in Surinam is weekly, once every two weeks in Gambia was documented. Results of bioassay in different parts of the world are varying. This difference was due to formulation of insecticide, species of *Anopheles*, susceptibility level of mosquito, the time of exposure, and texture of bednet, and type of test[15,16]. A comprehensive study showed that the mortality of *An. stephensi* against Olyset-Net was 97% on unwashed net and decreased to 9% on 20× washed one. Percent knockdown in 1 hour declined from 100% to 5% after 20 washes, whereas against PermaNet mortality was 94.9% and 90% on unwashed and 20× washed nets, respectively. There was no significant difference between 1 h knockdown time between the unwashed and 20× washed ones. PermaNet showed higher efficacy than the OlysetNet [17].

For coordinate large-scale field studies of Interceptor® and confirming its long lasting efficacy, longevity and fabric integrity, as a requirement for development of study at the hut and filed circumstances in the country is essential.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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