Original Article

Wash Resistance and Bioefficacy of Alpha-cypermethrin Long Lasting Impregnated Nets (LLIN-Interceptor®) against *Anopheles stephensi* using Tunnel Test

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Abstract

Background: The Long-lasing insecticide impregnated nets (LLINs) is considered as an effective tools for malaria vector control. The aim of this study was to evaluate the residual efficacy of alpha-cypermethrin long lasting impregnated nets (LLIN-Interceptor®) against *Anopheles stephensi* using tunnel test.
Methods: The wash-resistance of Interceptor® nets were assessed under laboratory conditions using tunnel test. Females of *An. stephensi* were released into the tunnel and then they were provided blood meals from guinea pigs. Bed nets were washed according to the standard procedure up to 20 times. The bioefficacy indicators such as inhibition of bloodmeal from experimental animal, knockdown, irritancy rate, survival rate, entry index and mortality were calculated.
Results: It induced 90–100% mortalities in the population of *An. stephensi* up to 15 washes. The KT_{50} values reduced from 73.47 to 26.30 minutes in unwashed in comparison to one washed, respectively. The mean of mortality rate of blood-feeding inhibition and entry indexes was reached to 91.6%±2.8, 87.0±3.4 and 24.9±2.8 respectively after 20 washing.
Conclusion: This net could provide a good personal protection against malaria vectors and could induce relatively high mortality, inhibit the blood-feeding as well as reduce the entry rates of female mosquitoes even after several washes.

Keywords: Alpha-cypermethrin, LLINs, *Anopheles stephensi*, Tunnel test

Introduction

According to the recent publication of WHO (2012) bednet impregnated with pyrethroids especially long lasting one is highly recommended for control of malaria. This intervention also is carrying out in Iran.

The southeastern part of the Iran remains as malaria-endemic area. Annual Parasite Incidence (API) was reported as 7 per 1,000 populations. The disease mainly occurs in three south-eastern provinces. In 2002, 68% of positive cases belonged to the Hormozghan, Sistan and Baluchistan and Kerman Provinces whereas in 2009 it increased to 90% (Raeisi et al. 2008).

There are several interventions to control malaria such as diagnosis and effective treatment of malaria cases and vector control, including the use of insecticide treated nets (ITNs), and indoor residual spraying (IRS) (Abai et al. 2008, Raeisi et al. 2008). An important innovation touring the past decade is the widespread introduction of insecticide-treated mosquito nets (ITNs and LLINs) for protection against malaria transmission.

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Various methods for mosquito control have been suggested by WHO. Pyrethroids are recommended for impregnation of mosquito nets due to high efficacy at low dosage, rapid knock-down effect and low mammalian toxicity (WHO 2007). Incorporation of pyrethroids into conventional netting fibers (polyester, polypropylene) is a new method for preparing the LLINs. α-cypermethrin is a cyano-group pyrethroid recommended for impregnation of bednet modeled on the natural defensive compound produced by chrysanthemums. The main challenge was to incorporate α-cypermethrin, in a polymer so that it would migrate to the surface at a steady rate over several years (Duchon et al. 2005). The WHO Pesticide Evaluation Scheme (WHOPES) recommended five long-lasting insecticidal nets (LLINs) for the prevention of malaria: Olyset®, PermaNet® 2.0, Netprotect®, Duranet® and Interceptor® (WHO 2005). Comparative efficacy of ITNs and LLINs had been carried out in order to optimize the different bioassay tests and calibration of technique in Iran (Vatandoost et al. 2006 a,b,c, Kayedi et al. 2008, Rafinejad et al. 2008, Vatandoost et al. 2009a,b).

The objective of this study was to determine the wash resistance of Interceptor® (LLINs) based on employing bioefficacy indicators using tunnel test device against the main malaria vector, *Anopheles stephensi*.

Materials and Methods

Long lasting insecticidal mosquito net (LLINs)

Alpha-cypermethrin, Interceptor® was provided by BASF Chemical Company, Ludwigshafen, Germany. This polyester mosquito net has 100 deniers. Alpha-cypermethrin at the dosage of 200 mg a.i./m^2 was mixed in a resin coating the netting fibers. The insecticide is progressively released from the resin and the net retains efficacy after repeated washings. The batches of 25×25 cm nets were provided for using in tunnel test between small compartment and large compartment. An untreated similar net type used for control.

Washing procedure

Net samples (25×25 cm) were individually introduced into one liter beakers containing 0.5 liter deionized water, with 2 g/L soap savon de Marseille "Le Chat®". The soap was added and fully dissolved just before introduction of net samples. Beakers were immediately placed into a water bath at 30 ºC and were shacked for 10 min at 155 rounds per min. The machine is the same as recommend by WHO (2005). The samples were then removed and rinsed twice for 10 minutes in clean, deionized water in the same shaking conditions. Nets were dried at room temperature and stored at 30 ºC in the dark. A piece of 25×25cm was used for tunnel test prior and after every washing (eg, 0x, 1x, 2x, 3x, 6x, 8x, 15x, 20x). This is following of WHO standard washing procedure (WHO 2005). The net was washed and dried once a week. The netting sample had nine holes with 1 cm diameter and the nets were fixed on a cardboard frame. The nets were replicated as appropriate number.

Mosquitoes

The Beech strain of *An. stephensi* which is susceptible to insecticides is used. The breeding was carried out at 29±1 ºC and 60±10% relative humidity at 16:8 h light-dark photoperiod. The larvae were fed with enriched wheat germ and the adults received a 10% sucrose solution. Guinea pigs were used for blood-feeding of female mosquitoes.

At each replicate of the biological efficacy of the nets, a total of 100 sugar-fed of 5–8 days females were used. The sucrose solution was picked up from the cage, 12 hour before starting the experiments. The female mosquitoes were introduced into the releasing chamber (big compartment) of the tunnel at 18:00 and then results were observed at 09:00 in the following day in the morning. The experiments were carried out at 28.0±1 ºC and 55.0±10% relative humidity.

Tunnel test

Laboratory tests were carried out in cubic glass tunnel device which simulated the experimental hut conditions, as described by Elissa et al. 1995, Chandre et al. 2000, Hougard et al. 2003, Corbel et al. 2004, WHO 2005, Duchon et al. 2007. The tunnel dimension was 25 cm in width, 25 cm in height and 60 cm in length. The width, height and length were respectively 25, 25 and 40 cm fitted with a metal cubic cage covered with untreated polyester netting for releasing of mosquitoes. The small compartment, 25x 25x 20 cm was designed for placing guinea pigs. The mosquitoes can entered into small compartment passing the 9 circulars of 1 cm diameter holes on both treated and untreated nets (Fig. 1). One separate tunnel with untreated netting was used as a control. Mosquitoes were free to fly in the tunnel. They also had a free choice contact with torn netting prior passing throughout the hole to reach the bait. Live mosquitoes were transferred into disposable cups with cotton pad of 5% sucrose solution.

Indicators and statistical analysis

The mosquitoes which were not able to fly and attached to the nets or corner of glass walls of the tunnel apparatus considered as knock-down. The knockdown rate was taken into consideration the number of unable mosquitoes to fly during 1-64 minutes after releasing in the compartments of tunnel apparatus. The probit analysis of Finney was employed based on knockdown data at logarithmic times (Finney 1971) for calculating KT50. The following morning, the live mosquitoes were recaptured using glass aspirator. They were transferred to a cupboard cups covered with net and maintained 24 hour for calculating the mortality rate. The dead mosquitoes were removed from each compartment after 15 hours and then scored separately at both big and small compartments. Overall mortality was measured by pooling the immediate and delayed mortalities. If the control mortality was between 5 and 20%, the percentage mortality was corrected using Abbott's formula (Abbott 1925). The inverse of the rate was calculated as the survival rate including both living blood-fed and unfed mosquitoes. The blood-feeding rate was defined as the number of mosquitoes which took a bloodmeal and may remain in small compartment or return to at the opposite side. The inhibition of blood feeding was assessed by comparing the proportion of blood-fed females (alive or dead) in treated or control tunnels. During the experiments, a proportion of bloodfed mosquitoes came back from the host chamber to releasing chamber was considered as returning rate. This phenomenon may be related to a behavioristic response to find a place having the greatest distance from treated nets. The proportion of mosquitoes (dead or alive and blood-fed or unfed) which passed through the netting was also recorded as the entry index. By comparison with the control group, any change in the entry index was considered as the indication for the repellent effect of the treated net. The significant differences between entry index, inhibition of blood feeding and mortalities at various washing conditions were analyzed using Pearson and Likelihood ratio Chisquare tests at Minitab 15.0 software.

Results

Knockdown rate

One hour knockdown rate of females to unwashed, up to 20-washed is shown in Table 1. The knockdown rates were always lower than mortality rates in the treatment group (Fig. 2). The knockdown rates had the decreasing trend from unwashed to 20 washes (Fig. 2). KT50s were 73.4, 26.3, 45.3, 72.4, 138.6, 90.0, 52.5, and 211.8 minutes respectively to different washing conditions and the confidence interval of KD50s are conjointly presented (Fig. 3).

Mortality tests

The total mortality rates ranged from 89.0 to 99.0% in unwashed and up to 15 times with the mean of 89.0±3.1%.The figures was ranging between 0 and 4.0% on serial washed untreated nets (Table 3). The mortality slightly drop to 75.0±4.3% at 20 washed treated net (Fig. 3). The 80.0% mortality rate of *An. stephensi* was indicated as a cut-off point for the decision making the reduction of alpha-cypermethrin bioefficacy (Duchon et al. 2005). The mortality rates of *An. stephensi* at releasing chamber were 75.0% and 90.0% on unwashed and one-washed, respectively, which correlated with related number mosquitoes which repelled and remained at big compartment (Table 3). The mortality rates in blood-fed and unfed are separately compared in control and treatment group.

Blood feeding indicators

Inhibition of blood-feeding was ranged between 80.0–99.0% in the treatment group, including unwashed and all nets which were washed 1–20 times (Table 4). A drop to 64.0% was considered on 20 washes. The inhibition of blood-feeding was negligible. It is varied from 0–10.0% for untreated netting. During this experiment, a total of 800 hungry females were tested on unwashed and all nets washed 1–15 times, 49 mosquitoes (6.1%) succeed to pass throughout the holes of treated nets toward the guinea pig in small chamber. They took blood and returned toward releasing chamber and survived after 24 hours of exposure. The blood feeding rate (individual protection) was reached to the highest level (36.0%) after 20-washing.

Survival rate

Survival rate of *An. stephensi* from washed to unwashed treatment were 1.0 to 11.0% (Table 4). This criteria was increased to 25.0% after 20-washing. The figure was 96.0 to 100% in the control group.

Entry index

The proportion of hungry mosquitoes that succeed passing (entry index) on unwashed and one-washed ranged between 25.0 to 10.0% .Taking a blood meal varied from 7.0 to 1.0%, whereas in the control group the entry index was 54.0 to 96.0% and 96.0 up to 100%, respectively. The variation of entry index and taking a blood-meal in the treatment group were 16.0–39.0% and 8.0–36.0%. The figures in the control group were 71.0–89.0% and 90.0–100% respectively during the test on 2-washed up to 20-washed nets (Table 3).

Individual and community protection rates

Individual protection and community rates were ranged between 80.0–99.0% and 91.0–99.0% respectively in unwashed and all nets washed for 1–15 times (Table 4). A drop to 64.0 and 79% were considered on 20 washes. The Individual protection and community rates were negligible and varied from 0–14.0% and 0–10% for untreated netting.

Irritancy effect

The Irritancy effect was ranged from 62.0–90.0% and 4.0–46.0% in treated and control groups (Table 3). The highest and lowest values of irritancy effect were 61.0±4.9% and 90.0±3.0% which for 20 and 1 washes respectively.

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Table 1. Comparison of knockdown and mortality rates of *An. stephensi* exposed to long lasting alpha-cypermethrin treated nets

Number of washes	Total mosquito tested	knockdown rate (%) after 1 hour at big compartment (Mortality rate after 24 h)	knockdown rate (%) after 1 hour at small compartment (Mortality rate after 24 h)	Total knockdown rate (%) after 1 hour (Mortality rate after 24 h)	Ratio of knockdown rate / Mortality rate
Unwashed	100	52 (75)	4 (24)	56 (99)	0.6
1	100	87 (90)	9 (9)	97 (99)	1.0
2	100	60 (73)	9 (18)	69 (91)	0.8
3	100	40 (60)	15 (29)	55 (89)	0.6
6	100	37 (84)	8 (11)	45 (95)	0.5
8	100	30 (83)	5 (13)	35 (96)	0.4
15	100	45 (74)	8 (15)	53 (89)	0.6
20	100	32 (56)	2 (19)	34 (75)	0.5

Table 2. Probit analysis on knockdown (KT_{50}) of *An. stephensi* and parameters of regression lines of KT_{50} at different washing conditions of long-lasting alpha-cypermethrin treated nets

Number of wash	A	B± SE	KT_{50} with 95% C.I.	χ 2 (heterogeneity) Calculated	Table (df)	*P*-value
0	-2.0608	1.1043±0.235	33.3574 73.4729 119.3313	21.216	16.7	<0.05
1	-2.7480	1.9350± 0.413	15.0470 26.3098 31.1217	41.625	16.7	<0.05
2	-2.7313	1.6496±0.286	27.9765 45.2653 58.4710	19.593	16.7	<0.05
3	-2.8246	1.5188±0.319	37.7958 72.4025 105.4589	20.885	16.7	<0.05
6	-1.0572	0.9605±0.117	83.9697 138.5827 298.6072	7.385	16.7	>0.05
8	-4.0753	2.0853±0.270	69.4992 90.0128 133.6082	1.625	16.7	>0.05
15	-3.3989	1.9756±0.189	43.4574 52.5295 66.9256	3.358	16.7	>0.05
20	-2.2114	0.9507±0.127	116.2165 211.8473 562.8679	3.989	16.7	>0.05

Fig. 1. Tunnel test apparatus

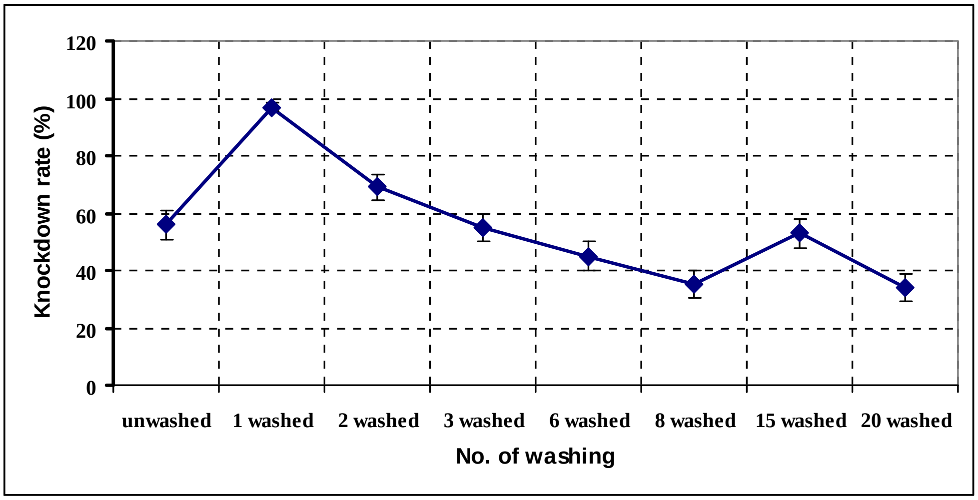

Fig. 2. Knock down rate of *An. stephensi* exposed to unwashed and repeated washed alpha-cypermethrin nets using tunnel test method

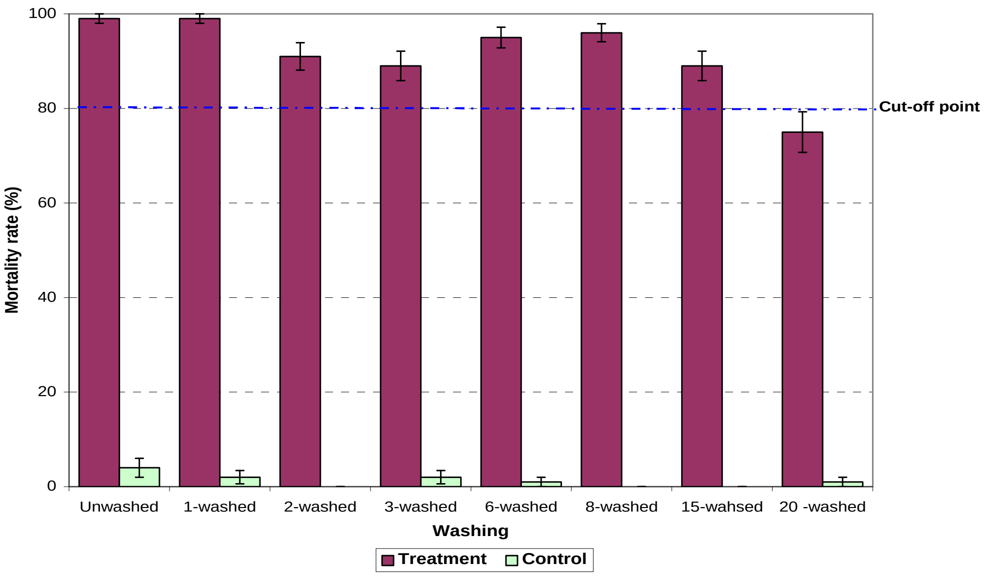

Fig. 3. Mortality rates of *An. stephensi* exposed to unwashed and serial washed long-lasting alpha-cypermethrin treated nets with control group

Table 3. Indicators for bioefficacy of alpha-cypermethrin treated nets (T) against *An. stephensi* at both big and small compartments of tunnel test apparatus compared to untreated netting as control group (C)

Number of washing	Type of test	Entry index % ± SE	Irritant effect (%) (at big compartment) ± SE	Ratio of mosquito dispersion at compartments (B/S ratio)	Mortality rate (%) at small compartment (Dead No.)	Mortality rate (%) at big compartment (Dead No.)	Total mortality rate (%) ± SE
0	T	25.0±4.3	75.0±4.3	3.0	96.0 (24)	100 (75)	99.0±1.0
	C	54.0±5.0	46.0±5.0	0.9	0 (0)	8.7 (4)	4.0±2.0
1	T	10.0±3.0	90.0± 3.0	9.0	90.0 (9)	100 (90)	99.0±1.0
	C	96.0±2.0	4.0±2.0	0.1	1.1 (1)	25 (1)	2.0±1.4
2	T	27.0±4.4	73.0±4.4	2.7	66.7 (18)	100 (73)	91.0±2.9
	C	80.0±4.0	20.0± 4.0	0.3	0 (0)	0 (0)	0
3	T	38.0±4.9	62.0±4.9	1.6	76.3 (29)	96.8 (60)	89.0±3.1
	C	77.0±4.2	23.0±4.2	0.3	0 (0)	8.7 (2)	2.0±1.4
6	T	16.0±3.7	84.0±3.7	5.2	68.8 (11)	100 (84)	95.0±2.2
	C	71.0±4.5	29.0±4.5	0.4	1.4 (1)	0 (0)	1.0±1.0
8	T	17.0±3.8	83.0±3.8	6.4	76.5 (13)	100 (83)	96.0±1.9
	C	76.0±4.2	24.0±4.3	0.3	0 (0)	0 (0)	0
15	T	21.0±4.1	79.0±4.1	4.9	71.4 (15)	93.7 (74)	89.0±3.1
	C	83.0±3.8	17.0±3.8	0.2	0 (0)	0 (0)	0
20	T	39.0±4.9	61.0±4.9	2.9	48.7 (19)	91.8 (56)	75.0±4.3
	C	89.0±3.1	11.0±3.1	0.1	0 (0)	9.1 (1)	1.0±1.0

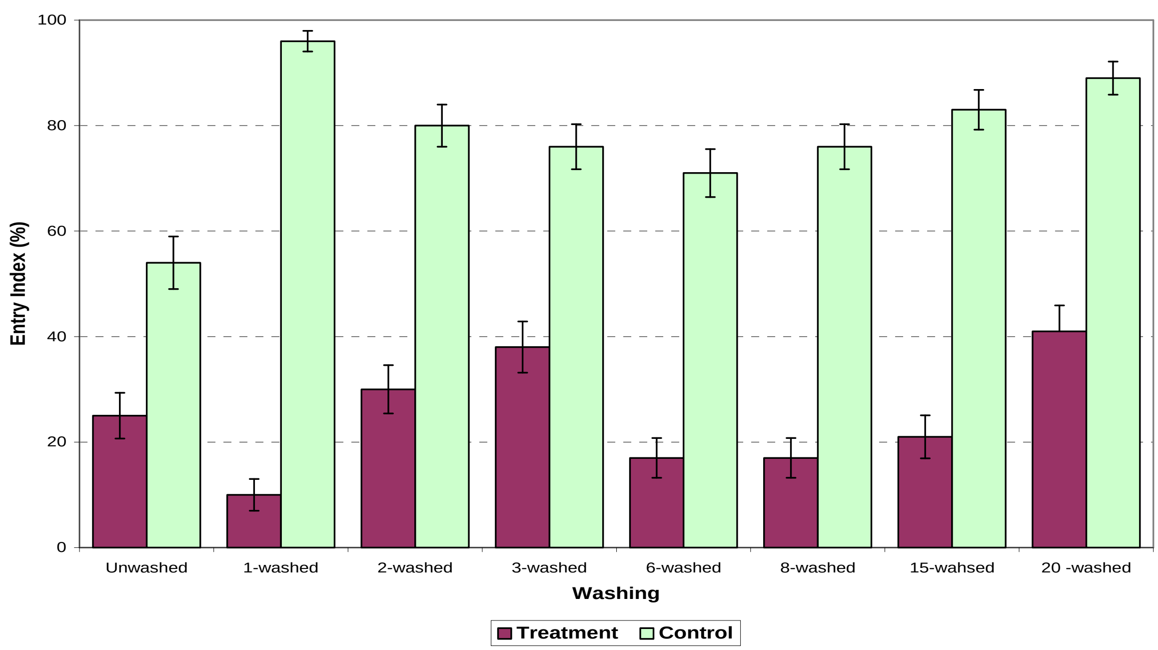

Fig. 4. Comparison of entry index of *An. stephensi* at compartment having long-lasting alpha-cypermethrin treated torn nets compared to untreated torn nets (control group)

Table 4. Bioefficacy indicators of *An. stephensi* exposed to alpha-cypermethrin treated nets at laboratory condition

N of nets washed	Type test	Total ntested	Small Compartment				Big Compartment				Individual protection rate (%) ± SE	Community protection rate (%) ± SE	Inhibition of Blood Feeding (%) ± SE	Mortality rate (%) ± SE	Survival rate (%) ± SE
			Alive (n)		Dead (n)		Alive (n)		Dead (n)						
			UF	FF	UF	FF	UF	FF	UF	FF					
0	T	100	0	1	18	6	0	0	75	0	93.0±2.6	99.0±1.0	99.0±2.5	99.0±1.0	1.0±1.0
	C	100	0	54	0	0	0	42	0	4	0	4.0±2.0	4.0±2.0	4.0±2.0	96.0±2.0
1	T	100	0	1	9	0	0	0	90	0	99.0±1.0	99.0±1.0	99.0±1.0	99.0±1.0	1.0±1.0
	C	100	0	95	1	0	2	1	1	0	4.0±2.0	4.0±2.0	0	2.0±1.4	98.0±1.4
2	T	100	0	9	15	3	0	0	70	3	85.0±3.6	91.0±2.9	85.0± 3.6	91.0±2.9	9.0±2.7
	C	100	0	80	0	0	0	20	0	0	0	0	0	0	100
3	T	100	0	9	29	0	2	0	60	0	91.0±2.9	91.0±2.9	91.0±2.9	89.0±3.1	11.0±3.1
	C	100	1	76	0	0	1	20	2	0	14.0± 3.5	4.0 ±2.0	4.0±2.0	2.0±1.4	98.0±1.4
6	T	100	0	5	9	2	0	0	83	1	92.0±2.7	95.0±2.2	92.0±2.7	95.0±2.2	5.0±2.2
	C	100	0	70	1	0	9	20	0	0	10.0±3.0	10.0±3.0	10.0±3.0	1.0±1.0	99.0±1.0
8	T	100	4	0	5	8	0	0	83	0	92.0±2.7	100	92.0±2.7	96.0±1.9	4.0±2.0
	C	100	0	76	0	0	2	22	0	0	2.0±1.4	2.0±1.4	2.0±1.4	0	100
15	T	100	1	5	0	15	5	0	74	0	80.0± 4.0	95.0±2.2	80.0± 4.0	89.0±3.1	11.0±3.1
	C	100	2	81	0	0	5	12	0	0	7.0±2.6	7.0±2.6	7.0±2.6	0	100
20	T	100	1	19	4	15	3	2	56	0	64.0±4.8	79.0±4.1	64.0±4.8	75.0±4.3	25.0±4.3
	C	100	0	89	0	0	7	3	1	0	8.0±2.7	8.0±2.7	8.0±2.7	1.0±1.0	99.0±1.0

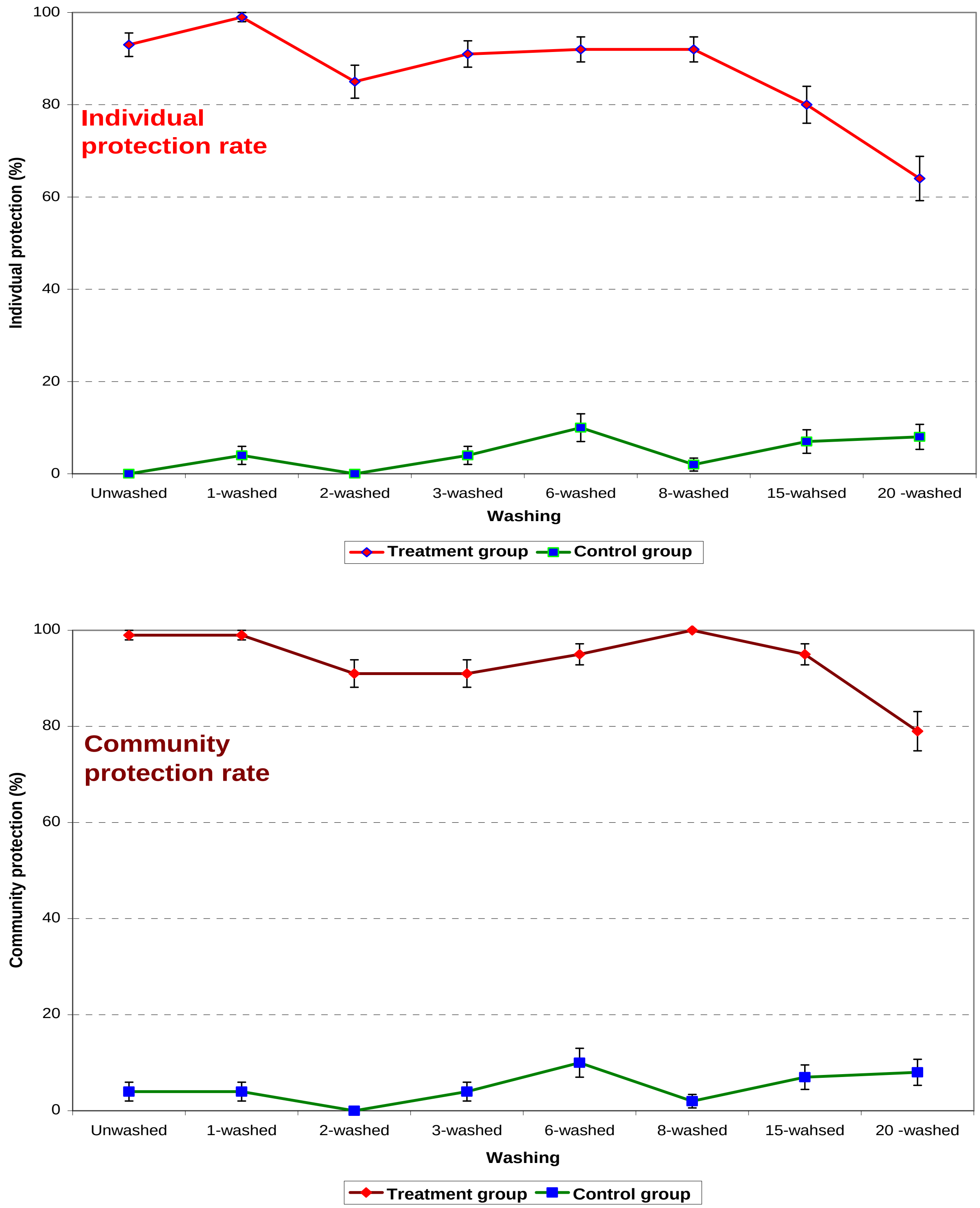

Fig. 5. Individual and community protection rates provided by long-lasting alpha-cypermethrin treated nets against *An. stephensi* compared to untreated netting

Discussion

There are several reports on the different aspect of malaria vector in Iran. The main malaria vectors in refractory malaria area are *An. culicifacies*, *An. stephensi*, *An. dthali*, *An. fluviatilis*, *An. superpictus*, and *An. pulcherrimus* which are known to be the malaria vectors (Vatandoost et al. 2002, Enayati et al. 2003, Naddaf et al. 2003, Davari et al. 2006, 2007, Doosti et al. 2006, 2007, Oshaghi et al. 2006a, b, Abai et al. 2008, Omrani et al. 2010, Vatandoost and Zahirnia 2010, Hanafi-Bojd et al. 2011, 2004a,b, 2005, 2006a,b,c,d, 2008, 2009a,b, 2010). *Aopheles sahacrovi* and *An. maculipennis* are considered as malaria vector in northern part of the country (Salari-Lak et al. 2002, Sedaghat et al. 2003a,b).

In this study the efficacy and wash-resistance of Interceptor® long-lasting nets (LLINs coated with alpha-cypermethrin) were assessed by employing bioefficacy indicators using tunnel test method. Several indicators were defined including knockdown rate, KD_{50}, KD_{90}, mortality tests, blood feeding indicators, assoli of blood feeding, survival rate, individual and community protection rates, irritancy effect and entry index. Each indicator reflect the conditions which may occurred when the mosquitoes exposed to long-lasting or conventional nets.

Our results indicated that KD_{50} and KD_{90} of mosquitoes during the exposure to long-lasting nets are considered as important indicator due to positive correlation with mortality rate of mosquitoes. The ratio of knockdown rate to the mortality rate showed a range of 0.4–1.0% at different washing conditions (Table 1). Knockdown rates had the decreasing trend from unwashed to 20 washes (Fig. 2). One hour knockdown rates did not correlate with 24 h mortality rates (Pearson's correlation coefficient= 0.485, P= 0.224). The KT_{50} trend showed an increase after progressive washes of long-lasting alpha-cypermethrin treated nets but there was an unexpected result in knockdown rates, which may be related to the presence of enough space at big compartment to avoid the mosquitoes to pick up from treated net. This variation in knockdown rate may be related to the activation of Fendozin®, a textile new auxiliary that binds the potent long-lasting alpha-cypermethrin treated nets in a special coating to the fibers of the net. The washing of treated nets was led to slowly releasing of alpha-cypermethrin and rapidly knocks down, kills or repels the mosquitoes as they come in contact with the treated nets. KT_{50} also increased after progressive washing.

The mortality of mosquitoes exposed to long-lasting alpha-cypermethrin treated nets which was washed 1 to 15 times, was high (89.0 to 99.0%). The mortality rate of *An. stephensi* that could pass overnight (15 h) from unwashed torn long-lasting alpha-cypermethrin treated nets toward to guinea pig place in the small compartment showed 96.0 and 90.0% mortality on unwashed and one-washed treated nets, which correlated with 25.0 and 10.0% of related entry index. The greater part of the mortality rates was occurred in the mosquito releasing chamber both on unwashed and all serial washes. It could be concluded that majority of the repelled mosquitoes were died without success in probing host placed at small compartment. Previous studies have found that a significant impact of washing (20 times) on long-lasting alpha-cypermethrin treated nets was observed on mortality (56%) but not on KD (97%) (Duchon et al. 2005). Our finding exhibited that the ratio of mosquitoes at the big compartment had always higher ranging 2.1–10 folds of small compartment both at unwashed nets and all washed. This ratio is always below 1.0% in control group. When the ratio of mosquitoes at big to small compartments was decreased, it could be expected the increasing of passing the mosquitoes through holes of treated nets toward the guinea pig as a blood feeding host placed

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at small compartment. A previous laboratory work on long-lasting alpha-cypermethrin treated nets against *An. stephensi* showed that after 3 washes, the mortality rate was 97.5% which concordance with 118.6 mg/m^2 as well as the mortality was 15% after 20 standard washes with the residue of 61.2 mg/m^2 (Vatandoost et al. 2011).

In the absence of insecticide, the inhibition of blood-feeding on host is negligible (0–10%). This is attributed on exposing of skin for mosquitoes as well as amount of chemical attractants (eg odors) of host. Our result indicated that inhibition of blood-feeding of mosquitoes was high (80–99%). A decreasing trend with increasing of washing times was observed. The previous studies also found the higher inhibition of blood-feeding of mosquitoes even after 30 standard washings of long-lasting alpha-cypermethrin treated nets. Also the blood feeding inhibition of mosquitoes in the alpha-cypermethrin treated houses was statistically significant ($P< 0.001$) (Sharma et al. 2010). During the other field trial on long-lasting alpha-cypermethrin treated nets, high mortality (above 95%) and high blood feeding inhibition (above 90%) were observed on *An. gambiae s.s* as well as for the other mosquito species. Also the latter study showed a blood-feeding increased in *An. gambiae* with the number of washes and was significantly lower (Duchon et al. 2005). Our findings showed that in control group, a significantly higher percentage of blood fed females was observed ($P< 0.001$). Our results may indicate that long-lasting alpha-cypermethrin treated nets (LLINs) have higher individual protection (>80%) up to 15 washes as well as higher community protection regardless of how many times the nets have been washed.

A previous work on long-lasting alpha-cypermethrin treated nets resulted lower entry index and higher mortality. The greatest reduction in blood feeding (4.8%) was achieved this insecticide (Mosha et al. 2008).

In a previous field trial, the personal protection of long-lasting alpha-cypermethrin treated which had been washed 20 times was almost 100%. However nets did not strongly reduce the entry rate of female mosquitoes, but kill almost all of them. These results suggested that the nets could have a strong mass effect of mosquito populations if used at large scale (Duchon et al. 2005).

Survival rates of *An. stephensi* which exposed to long-lasting alpha-cypermethrin treated nets showed an expected increasing trend toward to higher washing times, whereas this rate showed a nearly fixed trend in the control group. The entry index is reflecting the rate of mosquitoes that succeed in passing through torn nets try to enter the compartment where the host for blood feeding is available. The results from this study showed that the entry index was suppressed from the killing effect of long-lasting alpha-cypermethrin treated nets. There was a positive correlation between entry index and taking a blood meal (Pearson's correlation coefficient =0.784 and $P=0.02$). In a field study on long-lasting alpha-cypermethrin treated nets induced high mortality of *An. gambiae* that entered the hut. This fell significantly with the number of washes (0 washes: 93%, 20 washes: 79%, 30 washes: 66%) (Duchon et al. 2005). Our findings indicated that both entry index and blood-feeding rates increased trend from unwashed toward 20 washes. The entry index of mosquitoes that could pass overnight (15 h) through the holes of unwashed and one-washed treated nets was 25.0 and 10.0% which correlated with 24.0 and 9.0% mortality respectively (Pearson's correlation coefficient=0.682 and $P=0.01$). With the decrease of α long-lasting alpha-cypermethrin treated residue due to repeated washing, the entry index increased. The are several reports on the bioefficacy of bednet worldwide (Gimnig 2005, Kayedi et al. 2007, 2009, Gunasekaran and Vaidyanathan 2008, Oxborough et al. 2009).

The irritant effect of long-lasting alpha-cypermethrin treated nets was 75.0 and 90.0% on unwashed and one-washed nets. Irritancy effect of treated nets against *An. stephensi* was high. There is a significant reduction in entry rate of mosquitoes in alpha-cypermethrin treated houses in the absence of IRS ($P< 0.01$). Also the total excito-repellency rate of all the mosquitoes in houses with alpha-cypermethrin nets and untreated nets was 29.9 and 4.8% respectively (Sharma et al. 2010). During another field study on application of long-lasting alpha-cypermethrin treated bednets, the entry rates of the female mosquitoes were significantly reduced (15–30%) (Duchon et al. 2005). LLINs could be employed as a vector control intervention and the first field trials in India indicated that alpha-cypermethrin LLIN was effective in contributing to reducing the burden of malaria in the affected communities (Banek et al. 2010).

In conclusion, our laboratory trial on Interceptor® long-lasting nets (LLINs coated with alpha-cypermethrin) could provide a good personal protection against main malaria vector *An. stephensi* and could inhibit the blood-feeding of mosquitoes as well as induce relatively high mortality and reduce the entry rates of female mosquitoes.

We propose the hut and field trail on the effect of this kind of LLIN for measuring the impact and outcome indicators in the malarious area of south-eastern part of the country.

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