Original Article

High Performance Thin Layer Chromatography Analysis of Deltamethrin Residue on the Impregnated Bed Nets during a Leishmaniasis Control Program in Iran

SH Moosa-Kazemi[1], M Shayeghi[1], *MR Yaghoobi-Ershadi[1], H Vatandoost[1], MT Sadeghi[1], E Javadian[1], M Motabar[1], MR Hosseini[2], M Abtahi[1]

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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Abstract

Background: The control of leishmaniasis, a tropical neglected disease, has been concern of Iranian health authorities due to the increasing number of cases during the last two decades. The objective of this study was to determine deltamethrine residue on the impregnated bed nets using HPTLC technique in a leishmaniasis control program in Iran.

Methods: During this experimental study, a total of 130 small pieces of polyester netting were sewn to top, upper, and lower sides of some bed nets and then were impregnated with deltamethrin .The treated bed nets were distributed in Isfahan and Mashhad areas in April 2003. The samples were cut randomly after impregnation intervals. Deltamethrin was extracted using acetone from samples and the extract was applied for spotting onto plates. The plates were developed with n-hexane: ethyl acetate, 90+10(v/v), as a mobile phase in a Camage chamber. The qualifying of residue was observed in UV cabinet with λ=254 nm wavelength. Data were analyzed using SPSS version 11.5. and Stata Version 8. A three way ANOVA was used to compare the means of deltamethrin residue in each area, group and measuring time. The Kruskal-Wallis test was used to compare the means of residue for each of these factors with the control separately.

Results: The retardation factor of deltamethrin was calculated 0.50±0.02. The residues of deltamethrin persisted well on impregnated nets at least for 15 weeks after impregnation. No significant difference could be detected in the loss of residue of insecticide in comparison to measuring times and positions of sampling pieces on the bed nets in these areas.

Conclusion: Based on the results of the present study the use of HPTLC technique is recommended instead of other chromatographic methods for analysis of insecticide residue on the impregnated bed nets.

Keywords: *Leishmaniasis* control, Impregnated bed nets, Insecticides, Deltamethrin, HPTLC, Iran

Introduction

Cutaneous leishmaniasis due to *Leishmania major* (CLM) is still a great and increasing public health problem in many rural areas of 17 out of 30 provinces of Iran (Moin-Vaziri et al. 2007). Cutaneous leishmaniasis caused by *Leishmania. tropica* (CLT) is also a very old endemic disease in many foci and thousands of cases occur in large cities and small towns in the country (Yaghoobi-Ershadi et al. 2005). In recent years, considerable attention has been paid to vector control due to the increase of cases and resurgence of the diseases in some non-endemic areas of Iran. Bed nets impregnated with pyrethroid insecticides have been used successfully against phlebotomine sand flies worldwide (Yaghoobi-Ershadi et al. 2006, Moosa-Kazemi et al. 2007). Deltamethrin has proved to be one of the most

*Corresponding author: Prof Mohammad Reza Yaghoobi- Ershadi, E-mail: yaghoobi.reza@gmail.com

effective insecticides, especially under different climatic conditions, and it has already the most widely used insecticide for the effective impregnated bed nets for effective long term control of vector-borne diseases (Lengeler et al. 1998). Residue of insecticides on impregnated bed nets may be decreased during the transmission season of diseases and interrupt the process of control, so the quantifying of insecticides, residues as a quality control method on netting materials treatment is of great important (Bami 1981).

Application of chromatography for the separation, detection, and qualitative determination of pesticides, other organic chemicals, and related compounds are reviewed by several researchers. Analysis is involved in a variety of samples, such as food, agricultural and dairy products. There are many analytical methods such as gas chromatography (GC), gas-chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC). Quantitative HPTLC offers a viable alternative to gas chromatography or high performance liquid chromatography in terms of simplicity of operation, the availability, reproducibility, quantification at any time with changed conditions, calibration for quantitative analysis, cost effectiveness, and flexibility (Ludwick et al. 1977).

Although HPTLC method have been previously used for separation, detection and determination of residue of pesticides on soil, water, etc (Chen et al. 1996, Lekic et al. 2002, Haiqun et al. 2005, Sherma 2005), but there are few work using this method for quantification of pesticide on impregnated bed nets (IBS) in the world. HPTLC had not been used for chemical analysis of insecticides residues on impregnated bed nets during the vector-borne diseases control programs in Iran yet.

In this study, this method was utilized in the course of a cutaneous leishmaniasis control programme using IBs and Impregnated curtains (ICs) in the country to analyze the insecticide residue during 2003-2004.

Materials and Methods

The experimental investigation was conducted in the field and under laboratory circumstances. The field trial includes the selection of the study areas, bed net preparation, impregnation by deltamethrin, and bed net sampling. A laboratory study includes extraction, clean up, spotting, chromatogram development, detection and qualifying by UV, quantitative detection by CATS4 software of HPTLC.

The field study was conducted from June to September 2003 in two places, Habib- Abad in the rural district of Borkhar, Isfahan Province (32°38 N, 51°29 E) central Iran, and Shaghayegh located in Aab-o-Bargh area in the south west of Mashhad city (36°16 N, 59°35 E), Khorasan-e-Razavi Province, the northeast of the country. In 2003, the maximum and minimum mean monthly temperatures of Isfahan were 39.1 °C and -2.8 °C in July and December, respectively. The total annual rainfall was 124 mm with minimum 5.4 mm in March and Maximum 49.6 mm in January. The minimum mean monthly relative humidity was 12% (August and September) and the maximum was 89% (January). In the same period, the maximum and minimum mean monthly temperatures of Mashhad were 35.5 °C and -6 °C in July and December; respectively .The total annual rainfall was 276.3 mm. The minimum mean monthly relative humidity was 32% (July and August) and the maximum was 93% (January) in the city.

The white mosquito nets of multifilament polyester fiber 75-denier strength, 156-mesh size (12×13 holes/inch 2) in single (70×180× 150 cm), double (130×180×150 cm) and family sizes (190×180×150cm) were manufactured by a factory in Tehran. They were impregnated with deltamethrin (K-Othrine® Sc 5, OMS 1988, AgrEvo Marseille, France) at the target dose of 25 mg a.i/m^2, as described by WHO in 2002. A total of 130 small pieces in 5cm× 5cm size of netting of similar type were randomly sewn to some bed nets (top, upper, and

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lower side) and were impregnated by deltamethrin (WHO, 2002).The impregnation of the bed nets in both areas were carried out in April 2003. The nets and netting pieces received a code number and all the information such as the time of impregnation, size of bed nets were recorded in a special form. To assess the chemical residue analysis and quality of treatment, 3 to 10 pieces of the netting were cut randomly before use and after 3, 9, 12 and 15 weeks of impregnation in Borkhar area. The same numbers of the netting were sampled randomly before use and after 3, 8, 12 and 15 weeks of impregnation in Aab-o-Bargh area. Samples were put in small sterile vials individually and coded. The small vials were sealed by Para film and kept in 4° C temperature for the specific analysis (Gupta et al. 1998). A total of 130 samples (106 as deltamethrin-treated nets and 24 as untreated nets) were sent for chemical analysis to Pesticide Evaluation laboratory at the Department of Medical Entomology and Vector Control, Tehran University of Medical Sciences, Iran.

The standard analytical grade of deltamethrin (99%) was purchased from Accustandard Company (Tehran). Acetone, hexane, ethyl acetate, methanol (analytical-grade), and 20 cm ×20 cm aluminum foil backed silica gel 60 F254 HPTLC plates (layer thickness, 0.10-0.20 mm) were from Merck, Darmstadt, Germany. Linomat IV applicator, Chromatography tank, UV cabinet, TLC Scanner 4, an external PC running Wincats software (Version 1.1.2) were from Camag, Muttenz, Switzerland. Absorbance was measured at wavelength of λ= 254 nm by means of a deuterium lamp (Gupta et al. 1998, Sherma 2005).

For extraction of deltamethrin residue from fabrics, 20 ml pure acetone was added to each vials containing 25 cm^2 pieces of netting. The insecticide residue was extracted by 1 h shaking and then allowed to laid 1 h just before analysis to ensure extraction of a representative quantity of deltamethrin (Sherma 2005). The extracts were evaporated until the final solution reach to 1ml. Three replicate extractions were performed for each treatment. The spotting on a plate was performed by an applicator and capillary tubes. Volume of each spot was 10 µl and the distance between spots was 1 cm. Ten mg of deltamethrin standard was mixed with 90 ml of pure acetone solution to produce 10% concentration. Then the solution stored in glass-stopper bottles at 4 °C. Ten µl of extracted and standard solutions were spotted separately on a plate as a stationary phase. Different extract solutions from treatment and control netting samples as well as standard solution were used. After spotting and drying the spots, the plate was put in a vertical TLC chamber. A mixture of n-hexane: ethyl acetate, 90+10(v/v) was used as a solvent (mobile phase) (Sherma 2005). The plate was placed in the chamber saturated with vapors of the developing solvent. Then it was withdrawn from the chamber and the mobile phase was evaporated after 30 min. After drying, the plate was irradiated at 254 nm wavelength in UV cabinet and retardation factor (Rf) value was calculated. The retention factor, or Rf, is defined as the distance traveled by the compound divided by the distance traveled by the solvent (Sherma 2005). A retention factor is calculated and stored in the database for each of the samples in accordance with the following formula:

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

Finally, the quantitative of insecticide residue were scanned by TLC Scanner 4 (CAMAG), with 207 nm wavelength, using CATS4 software according to external standard calibration (Gupta et al. 1998, Sherma 2005).

The data were analyzed by using SPSS 11.5 and STATA 8.0. A three-way ANOVA test was used to compare the means of deltamethrin residue in each areas for three groups, top, upper, and lower sides of samples in five measuring times. The Kruskal-Wallis test was used to compare the means of deltamethrin re-

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sidue for each of these factors with the control samples separately. The amount of insecticide residue was considered significantly, when the *P* value was less than 0.05 (Zar 1996).

Results

The retardation factor (Rf) value of deltamethrin was 0.50±0.02. Table 1 shows the residue of deltamethrin in the net samples detected by HPTLC technique before use and 3, 9, 12 and 15 weeks after impregnation in Borkhar area. The average initial uptakes of the deltamethrin by top, up and lower sides of net samples were 26.50, 26.70, and 26.80 mg a.i./m^2, respectively. The mean uptake of insecticide was the greatest in the lower side sample for the first measuring time (26.70±1.63) followed by the second measuring time (25.89± 2.80) after 3 weeks of impregnation. Non-significant difference was observed in the residue analysis of top, up and lower side of samples in all sampling time using a Kruskal-Wallis test.

Table 2 presents the results of the residue analysis of the netting samples taken randomly from the impregnated bed nets before use and after 3, 8, 12 and 15 weeks of impregnation in Aab-o-Bargh area. The average initial uptake from top, up and lower side of the impregnated net samples indicated that deltamethrin suspension concentrate (DSC) formulations were 28.2, 27.80 and 27.11 mg a.i./m^2, respectively. The average and variation of insecticides residue were greatest for various side samples at 27.70± 0.44 of the first measuring time followed by top and lower sides of samples at 27.05±11.90, 27.01± 7.70 mg a.i./m^2 of the second sampling time, respectively. Non-significant difference was observed in the loss of chemical activity of top, up and lower sides of net materials, in each sampling time. Statistical analysis show that there are non-significant differences between two study areas, the situation of samples and duration of sampling in terms of deltamethrin residues by ANOVA test.

Table 1. Results of the TLC analysis of deltamethrin impregnated bed net samples (mg a.i/m^2) in Borkhar (Isfahan Province), Iran

Weeks after treatment	Control (mg/m^2)	Position of piece: Top				Upper side				Lower side			
		No.	Mean	SD	SE	No.	Mean	SD	SE	No.	Mean	SD	SE
Just after impregnation	0.0	2	26.50	3.80	2.70	2	26.70	1.50	1.10	2	26.80	1.50	1.10
3	0.0	5	25.61	3.83	1.7	4	25.52	2.74	1.37	4	26.56	10.91	5.45
9	0.0	5	25.24	8.59	3.54	3	25.258	3.27	1.88	3	24.82	2.50	1.44
12	0.0	4	25.00	5.21	2.60	3	25.17	1.39	0.8	3	24.69	4.69	2.71
15	0.0	3	24.58	3.08	1.77	3	25.12	3.23	1.86	3	24.48	1.269	0.73

Table 2. Results of the TLC analysis of deltamethrin impregnated bed net samples (mg a.i./m^2) in Mashhad (Khorasan-e-Razavi Province), Iran

Weeks After treatment	Control (mg/m^2)	Position of piece: Top				Upper side				Lower side			
		No.	Mean	SD	SE	No.	Mean	SD	SE	No.	Mean	SD	SE
Just after impregnation	0.0	2	28.20	16.40	11.60	2	27.8	12.10	8.60	3	27.10	9.8	8.02
3	0.0	5	27.05	26.60	11.90	3	26.88	10.11	5.84	3	27.01	13.34	7.70
8	0.0	4	26.06	13.45	6.72	4	26.10	3.91	1.95	4	25.54	8.80	4.40
12	0.0	3	25.46	3.24	1.87	3	25.37	3.96	2.29	4	25.45	3.58	1.00
15	0.0	3	24.60	2.21	1.28	4	25.16	10.78	5.39	3	24.58	2.45	1.42

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Discussion

This is the first report on the chemical analysis of the deltamethrin impregnated bed nets using HPTLC technique in relation to CLM and CLT endemic areas of Iran. There are several reports for quantification of pesticide on different samples (Ludwick et al. 1977, Haiqun Cao et al. 2005). Quantifying the insecticides deposit as a quality control in fabric treatment is very important. Deltamethrin was one of the photo stable synthetic pyrethroids to be developed for its stable residual activity, and specifically recommended by WHO to use for impregnation of mosquito nets. This insecticide with other alpha cyano compounds tend to be rather more toxic to insects and low toxicity to human, for this reason, remarkable small quantities are required on a net (Miller JE 1994, Alten et al. 2003). The susceptibility status of *Phlebotomus papatasi* and *Ph. sergenti* to this insecticide using WHO standard method indicated that the both species were susceptible to deltamethrin in Borkhar and Abo- Bargh areas (Yaghoobi-Ershadi et al. 2006, Moosa-Kazemi et al. 2007). As mentioned above, bed nets made of polyester with variation in size, weight, and different absorption capacities were used in this study. The difference between initial concentration of deltamethrin before used and after 3 weeks of impregnation of the bed nets in both areas was not significant. It is noteworthy that in the same field study, the initial uptake of the deltamethrin on bed net was high and dropped to nine-tenth of the original activity after 6 months (Alten et al. 2003).

In this study, the uptake of deltamethrin was closed the target dose after 15 weeks of impregnation in Borkhar and Aab-o-Bargh areas, where the uptake were 24.72±1.45 and 24.78±2.69 mg a.i./m^2, respectively. In the same study persistent of deltamethrin on treated nets was reported for 1 year, even with washed once or twice (Njunwak et al. 1991, Miller 1994,).

Bioassay test is simple and quick enough to use as a simple measure to show the effectiveness of impregnated nets and indirectly represents the amount of the deposit of deltamethrin on impregnated bed nets .It should be mentioned that in the same time a field study was carried out in the Borkhar area in 2003, and the mortality rate of P. *papatasi* (wild-caught, fed females) after 3 min of exposure time followed by 24 h recovery time was100%. The mortality of the species was 100% until the end of week 12. The mortality of control sand flies never exceeded 3.5% (Yaghoobi- Ershadi et al. 2006). At the same time, bioassay test showed that *P. sergenti* was susceptible to deltamethrin impregnated bed nets and this insecticide remained effective for 3 months and more in Abo-Bargh area (Moosa-Kazemi et al. 2007). Chemical residue analysis correlated well with bioassay results. The efficacy of deltamethrin-impregnated bed nets and curtains of this study confirmed findings of another similar recent trial which was carried out in the Colombia (Alexander B et al. 1995), However, Alexander et al. mentioned that the effectiveness of pyrethroid on the bed nets persisted at least 4 months (Alexander et al. 1995).

In the field study, the chemically detectable of deltamethrin was decreased approximately half the target dose by 3 months and to a third by 5 months (Jawara et al. 2001). Such a remarkable low residual activity is believed to be mainly due to the different netting or materials, poor dipping and impregnation techniques, dust and dirt in the area, as well as washing habits of residents, which affects the residue activity of insecticide.

In the other study, it was found that 14% of nets had been washed within one month and 76% within three months after impregnation (Jawara et al. 1998).Washing habits decreased insecticides concentration of treated bed nets (Snow et al. 1987, Lindsay et al. 1991, Lines 1996). It should be mentioned that at the same time, washing habits of residents were questioned through the projects and few of the impregnation bed nets were washed in Borkhar

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and Mashhad areas (Yaghoobi-Ershadi et al. 2006, Moosa-Kazemi et al. 2007).

In conclusion, the low cost and long persistence of deltamethrin on the impregnated bed nets and curtains (up to three months without washing) means that it can be used for preventing and controlling of the vector-borne diseases such as leishmaniasis in the other parts of Iran and neighboring countries. In the forthcoming control program the use of HPTLC technique and quality Control of IBs, ICs and LLITNs can be recommended instead of other chromatographic methods.

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