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**Evaluation of Permethrin Treated Clothing for Personal Protection Against
Phlebotomus papatasi (Diptera: Psychodidae)**

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Abstract: This study was performed to assess the efficacy of permethrin-impregnated cloth (0.125 mg permethrin [AI] cm⁻²) against natural population of *Phlebotomus papatasi* (Scopoli), which is the main vector of zoonotic cutaneous leishmaniasis, under field condition. Eight volunteers were selected for this study. Five of them put on the treated uniforms and the other three ones wore the untreated uniforms. All the subjects participated in the night biting tests for eight active nights between July and August 2004. In this study, the average number of biting among those who wore untreated uniforms (controls) was 1.75 sand flies bitings min⁻¹ person⁻¹ (105 h⁻¹) against 0.52 (31.2 h⁻¹) for volunteers who wore treated uniforms. The relative protection percent of treated uniforms, in comparison with untreated ones against *P. papatasi* (Scopoli), was about 70.3%. Although the technique of permethrin-impregnated clothes are provided considerable protection against sand flies biting, but it may be not acceptably decrease leishmaniasis, especially in endemic and hyperendemic areas. Therefore, in addition to permethrin-impregnated clothes protection, concurrently using of repellents on exposed skin is suggested.

Key words: Permethrin, *Phlebotomus papatasi*, leishmaniasis, personal protection, Iran

INTRODUCTION

Leishmaniasis is a major health problem worldwide. Its burden remains important: 88 countries, 350 million people at risk, 500,000 new cases of visceral leishmaniasis and 1-1.5 million cutaneous leishmaniasis per year (Desjeux, 2004). Leishmaniasis is still an important public health problem due to not only environmental risk factors such as massive migrations, urbanization, deforestation, new irrigation schemes, but also to individual risk factors: HIV (Leishmania-HIV co infection), malnutrition, genetic, etc. Leishmaniasis is part of those diseases which still requires improved control tools (Piscopo and Azzopardi, 2007; Desjeux, 2004). Many of people such as adventure, travelers, missionaries and soldiers have an increased risk for leishmaniasis (especially cutaneous leishmaniasis) (Schwartz *et al.*, 2006; Antinori *et al.*, 2005; Croft *et al.*, 2006).

More than 90% of cutaneous leishmaniasis cases occur in Iran, Afghanistan, Syria, Saudi Arabia, Brazil and Peru (Alvar *et al.*, 2006; Faulde *et al.*, 2006). It is one of the main health problems in Iran and many of Middle East countries (Yaghoobi-Ershadi *et al.*, 2004; Rassi *et al.*, 2006; Khan and Muneeb, 2005).

Cutaneous Leishmaniasis (CL) is caused by different species of *Leishmania* and transmitted by the bite of infected sand flies. *Phlebotomus papatasi* is vector of *Leishmania major* and phleboviruses. Among the many vector-borne diseases that are currently emerging worldwide, few are vaccine-preventable. The search for effective vaccines against leishmaniasis is still in progress (Piscopo and

Azzopari, 2007). Therefore vector control is one of the main ways to reduce this disease (Hepburn, 2003). For this reason, personal protective measures against vector constitute the first line of defense against arthropod bites and arthropod-borne disease.

Permethrin, a synthetic pyrethroid insecticide, has high level of potency against a wide range of arthropods, quick knockdown, low mammalian toxicity and stability (Schreck and Klin, 1989; Frances and Writz, 2005).

A Permethrin-treated military uniform at safe dose (0.125 mg cm^{-2}) has been known as an effective technique for protecting military personnel against hematophagous insects (Deparis *et al.*, 2004). Several laboratory and field researches have been done in many armies of the world to evaluate the effectiveness of permethrin-treated uniforms; the results have been satisfactory in most cases (Faulde *et al.*, 2003).

Permethrin-treated military uniforms is among the hygienic programs of many armies of the world and has also been introduced in some countries like France, as a vector control strategy and protection method for military personnel against insects (Deparis *et al.*, 2001).

The protection of permethrin treated uniforms against different species insects in various geographical conditions is dissimilar. Therefore, in this study, permethrin-treated military uniforms were evaluated against *Phlebotomus papatasi* (main vector of zoonotic cutaneous leishmaniasis in Iran).

MATERIALS AND METHODS

Chemical

The emulsifiable concentrate (EC) of permethrin (10%) (cis/trans isomers with 25/75% ration, respectively) manufactured by Bayer Ltd. (Germany).

Study Sites and Volunteers

This study was conducted in Iran between July and August 2004. Dadin village in Fars province was selected as the test site because of the high density of sand flies population and rarity of leishmaniasis and other vector-borne diseases and being the place of health research institute in this district and availability of human local volunteers.

Eight local male subjects were selected for wearing permethrin-treated and untreated uniforms (five as cases and three as controls). In order to decrease subject caused bias, they wore the uniforms in a revolving manner (subjects in eight nights wore all treated and untreated uniforms).

Subjects were 18-28 years old, age average of 19.5 years. Eight officers were also appointed as supervisors. The subjects were trained about the test time and the needed activities. The supervisors were also trained about the method of data registration in sheets.

Fabric Treatment

The uniforms were treated at $0.125 \text{ mg active ingredient cm}^{-2}$ of cloth with aqueous suspensions of permethrin from 10% Emulsifiable Concentrate (EC) formulation (cis/trans isomers with 25/75% ration, respectively). The concentrations of treating solutions of permethrin were determined based on the quality and absorption coefficient of uniforms' textile and also the area of uniforms. Then, treatment was done by dipping method. Sufficient liquid was used to saturate each article of clothing without runoff. Saturated fabrics were placed in plastic bags for 24 h to enhance liquid penetration. The uniforms were then removed from the bags, placed horizontally on aluminum foil and turned periodically to air dry without loss of permethrin from dripping. Before testing, the clothing was labeled and stored in fresh plastic bags (Schreck and Klin, 1989). The control uniforms were treated with water. The uniforms were kept in laboratory condition (temperature: 23-26°C, humidity: 30-40%).

Test Procedure

The subjects were selected among local people of the study village and trained about the test and obtained their consent in writing to be a volunteer in the study. They were asked not to use insect repellent or attractive materials like perfumes, alcohol, smoke, garlic, mint and other odorous materials. Subjects received the uniforms, half an hour before sunset. Five of the selected subjects wore the treated uniforms and three others wore the control untreated uniforms. Subjects sat in appropriate distance of each other (5-10 m). The control subjects sat in the same manner, in 50-100 m distance of case subjects. Field test was started at sun set. One supervisor was considered for each subject. The supervisors entered the night biting data in the record sheets provided. They did the task by direct watching of the biting point. Then they captured the sand flies using aspirators and transferred them into the cups. Meanwhile, subjects changed their place every half an hour to face new mosquitoes and prevent a fatigue or knockdown effect on mosquitoes because of repeated contacts with treated uniforms in the same areas (Schreck and Klin, 1989; EPA, 1999).

Night biting test was started at 19:30 h and ended at 01:00 h every night, when number of bitings decreased. The subjects ate their dinner before starting the test and did not have any activity during the test.

This night biting test was repeated for eight times in consecutive nights.

Statistical Analysis

For statistical analysis data were first transformed by $\sqrt{y+0.5}$ formula (y: number of bitings per min) and then Poisson distribution was used for statistical analysis of biting number in case and control groups. For comparing the means of biting on skin in treated and untreated groups, t-test was used. After statistical analysis, the means were retransformed to original units and converted to mosquito bites per min. Protection percent of treated uniforms was calculated using the following formula:

$$\text{Protection percent} = \frac{\text{Untreated biting} - \text{Treated biting}}{\text{Untreated biting}} \times 100$$

RESULTS AND DISCUSSION

The mean of air temperature and relative humidity during study was 27.4°C (20-33°C) and 60.1% (42-75%), respectively. The supervisors recorded all of bitings on subjects besides capturing the sand flies using hand aspirators. All sand flies captured in this study were identified *P. papatasi* (Scopoli). The mean of biting per person for treated and untreated uniforms was 31.2 h⁻¹ (31.2±1.82) and 105.0 h⁻¹ (105±5.65), respectively (Table 1). The mean of biting in untreated uniforms was significantly higher than treated uniforms (p<0.01). Comparison of treated and untreated uniforms showed that the protection percent of treated uniforms against *P. papatasi* biting was 70.3% (Table 1).

In this study, the percent protection of permethrin- impregnated uniforms against natural population of *P. papatasi* was observed to be around 70%. In others studies, with the same condition (in rural area), permethrin-treated uniforms provided 83 and 78.7% protection against several species of the *Culex* mosquitoes and *An. stephensi* (the main malaria vector in south of Iran), respectively (Khoobdel *et al.*, 2006). In another study permethrin-treated uniforms provided more than 89% protection against *Cx. pipines* in urban area in Tehran (capital of Iran) (Khoobdel *et al.*, 2005). Also in France soldiers, wearing impregnated uniforms, had more than 90% protection against ticks (Deparis *et al.*, 2001). Present study has showed the protection of permethrin-treated uniforms against *Ph. papatasi* was less than that of mosquitoes. Other study also demonstrated that sand flies are less sensitive than mosquitoes to knockdown during contact with permethrin treated fabric; however,

Table 1: Mean of percent protection of permethrin impregnated clothing against *P. papatasi*

Treatments	Sand flies bites per hour			
	On skin±SE	Through clothing	Overall	Protection (%)
Untreated	105.0±5.65	0.0	105.0	0.0
Treated	31.2±1.82	0.0	31.2	70.3

24 h mortality rates for sand flies were higher when compared with mosquitoes (Fryauff *et al.*, 1996). Also studies in African countries showed that using permethrin-impregnated curtains in Burkina Faso lead to complete elimination of endophilic sandfly species (Maroli *et al.*, 1991). The studies in Colombian soldiers showed wearing permethrin-impregnated uniforms decreased incidence of leishmaniasis (Soto *et al.*, 1995). In a study in Iran, wearing permethrin-impregnated uniforms could not significantly reduce of cutaneous leishmaniasis among soldiers in endemic area (Asillian *et al.*, 2002). It is to be noted that decrease in biting does not mean decrease in Leishmaniasis might have occurred just by one or few infected bites (Hepburn, 2003). Therefore, using permethrin-treated uniforms in endemic area may not be alone effective in preventing leishmaniasis (Deparis *et al.*, 2004).

The results of this study has indicated that permethrin-treated uniforms provided considerable protection against sand flies biting, but this protection is not complete (100%). Therefore, for complete protection the impregnated uniforms and repellents should simultaneously be used. It may be noted that in Alaska concurrent use of impregnated uniforms and DEET 35% in skin exposed, had 99% protection against *Culiseta impatiens* in the place which had the density of 1188 bites for man hour (Lillie *et al.*, 1988).

P. papatasi is widely spread in different geographical regions of Iran. Present study has been accomplished in a restricted rural area, south of Iran. Therefore this survey can be considered as a pilot study. For exact determination of protection percent of permethrin-treated fabrics against *P. papatasi*, complementary study in other regions of Iran is needed.

Although, the technique of permethrin-impregnated clothes are provided considerably protection against *P. papatasi* biting, but it may be not acceptably decreased leishmaniasis, especially in endemic and hyper endemic area. Therefore for completed of permethrin-impregnated clothes protection, concurrently using of repellents on exposed skin is suggested.

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