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An Epidemiological Study of Cutaneous Leishmaniasis Using Active Case Finding among Elementary School Students in Pakdasht, Southeast of Tehran, Iran 2013-2014

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ABSTRACT

Background: Cutaneous leishmaniasis is one of the prevalent health problems in tropical and subtropical areas including Iran. Therefore, it is essential to identify the prevalence and new foci of this disease in different regions of Iran. This study aimed to determine the epidemiology of cutaneous leishmaniasis by active case finding in elementary schools in Pakdasht city, Southeast of Tehran, Iran 2013-2014.

Methods: This cross sectional study was carried out on 4800 students from 60 elementary schools in Pakdasht during October 2013 to March 2014. After examining the students, some epidemiological data such as age, gender, date, anatomic location of the lesion(s) in the body, number and size of lesion(s), lesion type, and travel history to endemic areas was recorded in a checklist. Students with active lesions were examined using parasitological and molecular methods (PCR).

Results: Totally, 31 students (0.64%) had leishmaniasis, of which 15 (0.31%, 95% CI, 0.15%, 0.47%) had active lesions and 16 (0.33%, 95% CI, 0.17%, 0.49%) had leishmaniasis scars. Molecular testing showed that *Leishmania major* was the causative agent of leishmaniasis in all patients with active lesion. The highest frequency of cutaneous leishmaniasis was found in the age group 10-12 years (P=0.03). There was a significant difference between the frequency of the disease in different months (P=0.04) and the anatomic location of the lesion/scar (P=0.04). Moreover, t-test revealed that there was a significant relationship between number of lesions/scars and the age group (P<0.001).

Conclusions: The leishmanial type diagnosed in all patients was *L. major*. This finding suggests that Pakdasht might be a new focus for zoonotic cutaneous leishmaniasis.

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Introduction

In the present era, despite the remarkable development of science and considerable progress in controlling infectious diseases, parasitic disorders are still considered a major health problem. Leishmaniasis is a parasitic disease considered as a public health problem in most countries, especially in tropical and subtropical regions of the world. This disease is caused by a kind of protozoa called *Leishmania* which is very important in developing countries such as Iran[1]. According to WHO, about 10 to 50 million people are infected worldwide and 367 million people in 88 countries are at risk of this disease. Annual incidence of human leishmaniasis is approximately 1.5 to 2 million[2-4]. Most cases of cutaneous leishmaniasis (CL) in the Middle East are reported from Afghanistan, Libya, Iraq, Iran, Jordan, Morocco, Saudi Arabia, Syria, Yemen, and Palestine[2]. Considering the importance of this disease, WHO has introduced it as one of six main diseases of tropical and subtropical areas[5].

Clinically, there are two types of CL in Iran, rural wet type and urban dry type. Rural CL is a zoonosis, while the urban type is anthroponotic. Although statistics show that about 20000 people get infected every year in Iran, researchers believe that the actual cases are 4 to 5 times more and not now a days it is one of the most important parasitic diseases in Iran[6].

Combating this disease has always been taken into huge consideration in national plans of Iran; despite national and international efforts and investments, this disease has not been controlled, but rather has appeared in new foci and has become more prevalent throughout the country. To select the appropriate method to combat this disease and to increase the

success of control programs, epidemiological characteristics of the disease must be determined[7,8]. Since it needs a long treatment period and, in some cases, treatment dose not result in complete recovery and some scars appear on uncovered parts of the body like hands and faces, this disease may cause mental discomfort in people. Thus, studying this disease is necessary to prevent it.

Some studies have been conducted in different parts of Iran on the epidemiology of CL in schools. Aflatoonian and Sharifi studied epidemiology of CL using active surveillance method in Bam[9] and Asgari Nezhad et al. studied epidemiology of CL among students of Dehloran[10].

Due to the increasing reports of the CL in this region over the past few years[11], and because of its proximity to Tehran, Pakdasht faces many immigrants mostly from eastern provinces of Iran. As a large number of students study in the elementary schools of this city and statistics obtained from previous studies reflect high prevalence of this disease among children[9,10], this research was carried out in 2013-2014 to study the epidemiology of CL in elementary schools of Pakdasht.

Methods

Participants

A total sample size consisted of 4800 students from 60 primary schools selected using random cluster sampling method. That is, the city and the rural areas of Pakdasht each was first divided into three districts and then 10 schools were selected from each district randomly; then 4800 students were selected from all 60 schools.

Study area

This descriptive-analytical study was conducted in 2013-14 (October to March) in Pakdasht located in the southeast of Tehran and covers an area of approximately 750 km^2. Pakdasht town is center of Pakdasht city. Geographically, it lies at longitude 51° east, at latitude 35° north, and at altitude of 1020 meters. It borders Alborz and Damavand mountain ranges in the north; Tehran and Rey cities in the west; central part of Varamin and Gharchak in the south; and Semnan province and Garmsar township in the east (Figure 1). Pakdasht has an arid climate with a little cold winters; maximum and minimum temperature are 47 ° and minus 10° Celsius, respectively.

Figure 1: Location of Tehran and Pakdasht within Iran

Examination

The study was approved by the Ethics Committee of Hamadan University of Medical Sciences. After obtaining the formal permission from the Education Department, all students were examined for sore or scar. With the help of the health teachers, uncovered parts of the body of all students were examined regarding new and active lesion(s) or old lesion(s) and scar(s). Students were also asked about presence of any lesions and scars on their bodies; results were recorded in a checklist. In addition, other necessary information including age, gender, address, date, lesion type, number and size of lesion(s), anatomic location of lesion(s) on the body, and history of travel to endemic areas was recorded. After examination, students with active lesions were referred to Shahid Ashrafi Esfahani Health and Treatment Center for further diagnosis.

Parasitology and laboratory methods

Triplicate smears were prepared from the exudate obtained by a minute incision made at the margin of the lesion with a sterile blunt scalpel No. 11, fixed by absolute methanol, stained using Giemsa (5%) stain, and checked under a light microscope with magnification 1000X in search for Leishman bodies.

DNA extraction

The specimens of all suspected patients were added to 200 µl lysis buffer (1 mM EDTA pH 8, 50 mM Tris-HCl buffer pH 7.6, 1% (v/v) Tween 20) and 8.5 µl of a proteinase K solution (19 µg/ml), at 55°C for 3 h[12]. The lysate was extracted with 200 µl of a phenol: chloroform: isoamyl-alcohol mixture (25:24:1, by volume). After centrifugation at 6000 g for 10 min, the upper aqueous phase was transferred to a new micro-tube and DNA was precipitated using cold absolute ethanol, resuspended in 50 micro liters of sterile double distilled water and stored at -20 °C until use.

PCR amplification

The DNA was amplified in 25 ml of reaction mixture consisting of 1 unit taq DNA polymerase (CinnaGen, Tehran, Iran), 200 ml of each deoxynucleoside-triphosphate (CinnaGen, Tehran, Iran), 1.5 mM $MgCl_2$, 1 µM LINR4, 1 µM LIN17 Primers, 200 pg of DNA and 2.5 ml of PCR buffer[13]. The forward LINR4 (5′-GGG GTT GGT GTA AAA TAG GG-3′) and reverse LIN17 (5′- TTT GAA CGG GAT TTC TG-3′) primers anneal within the conserved area of the minicircle kDNA which comprised the conserved sequence blocks 3 and 2, respectively[12]. The mixture was amplified in a CG1-96 thermo cycler (Corbett Research, Sydney, Australia) set for 5 min at 94 °C, followed by 30 cycles, each at 94 °C for 30 seconds, 52 °C for 30 seconds and 72 °C for 1 min, and then a final extension at 72 °C for 5 min. A 5 µl of the final PCR product was run on 1.5% agarose gel containing ethidium bromide, and visualized under ultraviolet light. The parasites were identified by comparison to reference strains of *L. tropica* (MHOM/IR/89/ARA2) and *L. major* (MHOM/IR/54/LV39).

Statistical analysis

Analysis of the data was made by SPSS (version 16) software, using the chi-square (X^2) or Fisher's exact test for qualitative variables and *t*-test for number of lesions/scars in different groups. A significance level of $P<0.05$ was selected.

Results

Totally, 4800 students were examined in 60 schools, of them 2392 (49.8%) students were male and the remaining 2408 (50.2%) were female. Frequency of active lesion was 0.31% (15 subjects) (95% CI, 0.15%, 0.47%) among all students; 53.3% (n=8) were girls and 46.7% (n=7) were boys. No significant difference was observed between two groups using chi-square test (*P*=0.806). Moreover, frequency of scar was 0.33% (16 students) (95% CI, 0.17%, 0.49%) with girls and boys having the same share. Of all subjects, 3837 students lived in urban areas and 963 ones in rural regions. Of all 31 students with active lesions and scars, only 3 students lived in rural areas and the remaining 28 ones lived in urban areas.

To recognize *Leishmania* parasites obtained from lesions, 18 Giemsa-stained samples taken from students who were suspected of having cutaneous leishmaniasis were examined using PCR. It was shown that 15 subjects whose parasitological tests were positive had positive results regarding molecular tests and 3 had negative results. PCR method recognized all 15 samples as *L. major* (Figure 2).

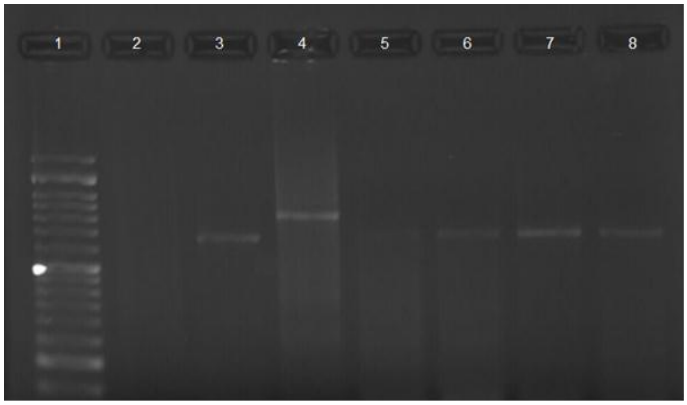

Figure 2: Agarose gel electrophoresis of *Leishmania* isolates. Lane 1, DNA size marker 100bp; lane 2, negative control; lane 3, *L. major* (positive control 650 bp); lane 4, *L. tropica* (positive control 760 bp); lanes 5, 6, 7 & 8 *L. major* isolates obtained from skin lesions of the patients.

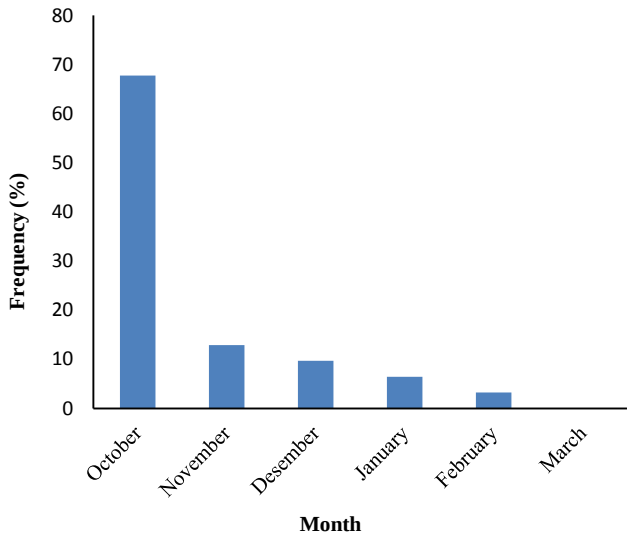

Figure 3: Prevalence of disease in different months of the year

The highest frequency of this disease was found in the age group 10-12 years (23 patients, 74.2%) and the lowest rate was observed in the age group of 7-9 years (8 patients, 25.8%); a significant difference was observed (*P*=0.04) (Table 1).

Table 1: The prevalence of cutaneous leishmaniasis in different age groups and sexes of school children

Age group (yr)	Female, n=16	Male, n=15	Total
7-9	4	4	8
10-12	12	11	23

The majority of cases were reported in October; statistical test showed a significant difference between the frequency of disease during different months of the year (*P*=0.03) (Figure 3).

Most of the lesions/scars were located on the feet of the patients (10 patients, 32.2%) and least were found on the trunk of the patients (3 patients, 9.6%), which it was statistically significant (*P*=0.04) (Figure 4).

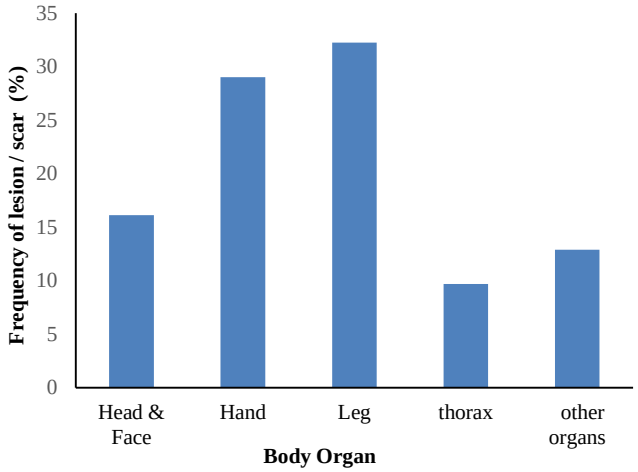

Figure 4: Location of lesion and scar on the body of school children

In total, 42% of patients had one lesion, 19% had two lesions and 39% had three or more lesions. The number of lesions/scars was significantly higher in females compared to males (*P*<0.001). Moreover, *t*-test results revealed that there was also a significant relationship between number of lesions/scars and the age group (*P*<0.001).

Discussion

This study suggests that Pakdasht could be a new focus for zoonotic cutaneous leishmaniasis (ZCL) near Tehran metropolis. This area might act as a focal point in transmission of the disease to the capital.

One of the most important parameters affecting the cutaneous leishmaniasis is the climate, which is a function of different conditions like seasons and months. The results of this survey indicated that the prevalence of the disease is higher in autumn, especially in October. This could be related to the features of disease vector. *Phlebotomus papatasi* is the main vector of ZCL in Iran. Since the highest activity of sandflies is seen in August and September, and due to the short incubation period of ZCL, lesions are usually formed in early autumn. Based on the results of some studies carried out by Amraee et al.[14] and Ramezani et al.[6], the highest prevalence of CL is in September and October; they are in line with the results of the present study.

Another factor influencing the prevalence of cutaneous leishmaniasis is traveling to endemic areas. Areas such as Isfahan[15], Kashan[16], Mazandaran[17], and Mashhad[18] are endemic areas of cutaneous leishmaniasis. In this study, the travel history of patients before the onset of disease was also

studied. The majority of patients had travel history to endemic areas such of Sabzevar and Kashan.

In the present study, there was a significant difference between frequency of lesions/scars in in various parts of the body (P=0.04). Lesions/scars were mostly found on the patients' feet, hands and face, whereas trunk had the lowest number of lesions/scars. Since sandflies cannot bite people through their clothes, so they are often attracted to the uncovered parts of the body and feed the blood there. Thus, the lesions are mainly found on the hands, feet and face. The present study is in agreement with the results of studies carried out by Nazari et al.[19], Youssefi et al.[17], Talari et al.[20] and Yaghoobi-Ershadi et al.[21], which reported that feet and hands had the highest rate of infection.

This study also showed significant differences between frequency of the disease among various age groups (P=0.03) and number of lesions/scars in different age groups (P<0.001); that is, the age group 10-12 years had the highest infection rate and the highest number of lesions/scars. These results are in line with the studies conducted by Asgari Nezhad et al.[10] and Aflatoonian and Sharifi[9].

Of 15 patients with active lesions, 46.6% were male and 53.3% were female. Like results of this survey, studies conducted by Gonzalez et al.[22], Kassiri et al.[23], Mohajeri et al.[24], Yaghoobi-Ershadi et al.[21] and Maghsood et al.[11] found also no significant difference in the frequency of CL in both sexes. But unlike the studies by Aflatoonian and Sharifi[9] and Nazari et al.[19], the present study showed a significant relationship between gender and number of lesions.

In this research, PCR test showed that all *Leishmania* parasites taken from patients were of *L. major* type. Using PCR, Mohajeri et al.[24] showed that *L. major* was the dominant type in Sabzevar. More importantly, 46.7% of patients with CL had traveled to Sabzevar. In a study conducted by Nekouie et al.[25] in Aberdezh, Varamin (located near Pakdasht), the dominant sandfly was *Phlebotomus* papatasi, 22% of which were infected with *Leishmania* promastigotes.

The limitation of this study was the examination of the female students' body. So, they were just asked for the presence of any lesions and scars on their bodies. The main strong point of the study lies in carrying out the molecular diagnostic method for accurate determination of the parasite species.

Conclusions

This study showed that Pakdasht might be a new focus of ZCL for people living there as well as for travelers or seasonal workers from/to Tehran. Since the leishmanial type recognized in Pakdasht was *L. major*, it is suggested that further studies should be carried out to recognize probable reservoirs and sandfly vectors of the disease in the region. This report recommends a comprehensive program for control of the disease and prevent the formation of a new active focus of zoonotic cutaneous leishmaniasis near the capital of Iran.

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Conflict of interest statement

The authors have no conflict of interest.

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