









Epidemiological Survey of Cutaneous Leishmaniasis in Mazandaran Province, Northern Iran

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A-R-T-I-C-L-E-I-N-F-O

Article Notes:

Received: Oct 07, 2019

Received in revised form:
Nov 03, 2019

Accepted: Nov 09, 2019

Available Online: Dec 10, 2019

Keywords:

Cutaneous leishmaniasis
Epidemiology
Iran

A-B-S-T-R-A-C-T

Background & Aims of the Study: Cutaneous leishmaniasis (CL) is considered one of the most important human vector-borne diseases in Iran. The current study aimed to determine some epidemiological aspects of cutaneous leishmaniasis in Mazandaran, Northern Iran.

Materials and Methods: This descriptive study was conducted using the epidemiological data, including demographic and clinical features collected from 379 patients diagnosed with leishmaniasis in health centers affiliated to the Deputy for Health Center of Mazandaran University of Medical Sciences within 2009-2017. The disease was diagnosed based on clinical information and direct microscopic examination of the samples. Data were analyzed in SPSS software using nonparametric Kruskal-Wallis and Mann-Whitney U tests.

Results: A total number of 379 individuals were diagnosed with CL during a 9-year period. Considering the population in Mazandaran, the incidence rate was reported as 1.27 per 100,000 population. It was also revealed that most of the cases of CL (n=279; 73.6%) were males and the majority of patients (n=137; 36.1%) were in the 20-29 age group. In addition, the majority of the ulcers were observed in hand (n=152; 26.7%) and foot (n=129; 22.6%), respectively. Moreover, just one ulcer was spotted in most of the patients (n=143; 37.7%). As the final note, the incidence was at the highest level during autumn in November (n=61; 16.1%).

Conclusion: As evidenced by the obtained results, cutaneous leishmaniasis poses a daunting challenge to the public health in Mazandaran. This disease can be attributed to several factors, including agricultural activities, animal husbandry, and frequent national and international holidaymakers' visits to the province. With this background in mind, the results of the current study could contribute greatly to the effective control of this disease.

Please cite this article as: Ghavibazou L, Hosseini-Vasoukolaei N, Enayati AA, Akhavan AA, Motevalli Haghi F, Arabi M, Fazeli-Dinan M, Shemshadian A. Epidemiological Survey of Cutaneous Leishmaniasis in Mazandaran Province, Northern Iran. Arch Hyg Sci 2019;8(3):178-186

Background

Leishmaniasis is a complex tropical disease with three forms of cutaneous, visceral, and mucocutaneous (1, 2). This disease is regarded as one of the eight important parasitic diseases in tropical areas of the world. Approximately 350 million people are at the risk of contracting this disease, about 12 million are believed to be infected and an estimated 2 million new cases of leishmaniasis are reported to occur annually in the world (2). The global burden of cutaneous and visceral leishmaniasis was estimated at 3,317,000 in 2010 (3).

This disease is caused by the kinetoplastid protozoa which belongs to the *Leishmania* genus transmitted through the bite of the phlebotomine sand fly species (1, 2). Out of 700 species of sand flies, only 10% have the ability to transmit 30 species of *Leishmania* (4).

Cutaneous leishmaniasis (CL) as the most characteristic form of the disease involves 1-1.5 million cases annually on a par with 50.75% of new cases (5). In Iran, two forms of cutaneous and visceral leishmaniasis have been observed; however, no evidence has pointed to the existence of mucocutaneous leishmaniasis (6). The incidence rate of CL in Iran increased from 20 per 100,000 in 2001 to 35 and 27 per 100,000 population in 2006 and 2011, respectively (3,7). Two forms of cutaneous leishmaniasis are reported in Iran, including Zoonotic Cutaneous Leishmaniasis (ZCL) caused by *Leishmania major* and Anthroponotic Cutaneous Leishmaniasis (ACL) emerged from *Leishmania tropica* (8, 9). Reservoirs of the ZCL are rodents belonging to the family of Gerbilidae, while human beings are the main reservoirs of ACL. The main vectors of ZCL and ACL are *Phlebotomus papatasi* and *Phlebotomus sergenti*, respectively (8, 9).

Iran Centers for Disease Control and Prevention (CDC) reported about 20,000 new cases of cutaneous leishmaniasis in most

provinces in Iran. Nonetheless, the actual incidence of the disease is believed to be 4-5 times higher [5]. This underreporting can be ascribed to diverse reasons, including non-referral of a high proportion of patients to health centers, especially in deprived areas, various diagnosis problems, and low sensitivity of common diagnostic procedures in the laboratory (10).

Cutaneous leishmaniasis is one of the most important health problems in many provinces in Iran and it has been reported that 17 out of 31 provinces of Iran are the endemic foci of ZCL, especially in rural areas. Considering the existence of endemic foci of CL in east and west of Mazandaran and due to the absence of comprehensive studies on leishmaniasis in northern Iran, the present study was conducted to provide a comprehensive overview of the epidemiology of this disease in Mazandaran. The current study aimed to investigate all aspects of the epidemiology of the disease, including the examination of patients, the rate and nature of the disease, determination of various causative agents and vectors in the development of different forms of the disease during 2009-2017 in Mazandaran, Northern Iran.

Materials & Methods

Mazandaran province is extended over an area of about 24,000 square kilometers ranging from 47° to 38° 5' North latitude and 50° 34' to 56° 14' East latitude of the Greenwich Meridian. Mazandaran is located on the southern coast of the Caspian Sea and boarded by Russia (across the sea), Golestan, Semnan, Tehran, Alborz, Qazvin, and Gilan provinces. Based on the latest divisions of the country, this province now has 22 townships with Sari being its capital. People in rural areas of Mazandaran usually adopt agriculture and horticulture as their main occupations. According to the topographic characteristics, Mazandaran can be divided into

two parts, namely coastal plains with temperate Caspian climate and mountainous areas with mountainous climates (Figure 1).

This descriptive study was performed on a total number of 379 patients with cutaneous leishmaniasis who were referred to health centers, clinics, outpatient clinics, and hospitals affiliated to the Deputy for Health of Mazandaran University of Medical Sciences during 2009-2017. Smears were prepared from all of the patients, were fixed in methanol for 5 min and were then stained with Giemsa for 30 min. The prepared smears were examined microscopically for the presence of amastigote form of *Leishmania*. After being diagnosed with the disease, the patients' epidemiological information was recorded in a patient-specific form and they were subsequently treated according to the national guideline for cutaneous leishmaniasis case management.

Required demographic and clinical data were registered and the relevance of these factors to disease incidence was analyzed. These factors entailed age, sex, nationality, place of residence, type and number of lesions,

location of the lesion on the body, month of infection, treatment failure, clinical resistance, and discontinued treatment. The population of Mazandaran is reported as 3,283,582 based on the latest population census (2016). The disease rate in the province was calculated based on the number of CL diagnosed patients in the 9-year period. The frequency of patients was analyzed and reported based on gender, location of the lesion, occupation, nationality, age, travel history, and the form of CL.

Before the commencement of the study, the approval was obtained from the Ethics Committee of Mazandaran University of Medical Sciences, Mazandaran, Iran (No.IR.MAZUMS.REC.1397.2836). The patients' data were collected, analyzed and reported unanimously. Thereafter, the data were analyzed in SPSS software (version 20). The nonparametric Kruskal-Wallis statistical test was used for the comparison of the data sets of more than two groups, whereas the data sets of two groups were compared using the nonparametric Mann-Whitney U test. A P-value less than 0.05 was considered statistically significant.

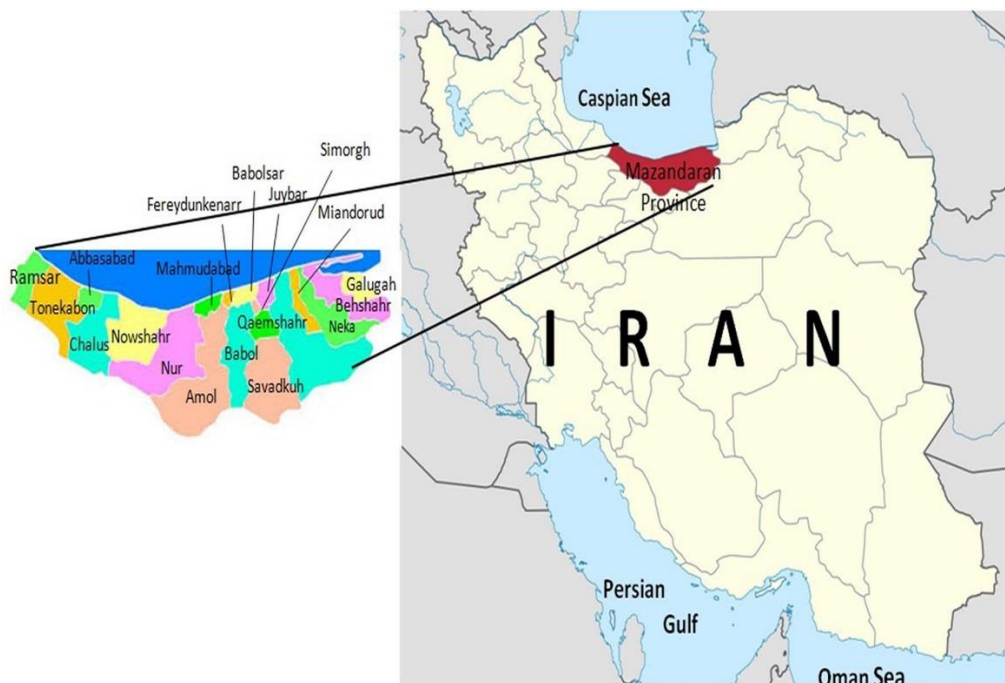


Figure 1) Status of study area in Mazandaran Province, 2009-2017

Results

The epidemiological data obtained from the patients diagnosed with CL were recorded at the health centers of Mazandaran from 2009 to 2017. The demographic and clinical characteristics of the patients are presented in the current study. In general, a total number of 379 individuals were diagnosed with CL in the present study. The incidence rate of CL varied during different years of the study. The average incidence rate of the disease during all 7 years was reported as 1.27 per 100,000 people. It was 0.6 in 2009, 2.01 in 2010, 1.43 in 2011, 1.7 in 2012, 1.3 in 2013, 1.18 in 2014, 1.06 in 2015, 1.43 in 2016, and 0.79 in 2017 per 100,000 population.

As depicted in Table 1, the distribution of the cases by gender is statistically significant. In this regard, 279 patients (73.6 %) were males and 100 cases (26.4 %) were reported to be females ($P=0.0001$). In addition, the frequency of the cases of CL was different based on the age groups. Most of the diagnosed cases were at 20-29 age group ($n=13$; 36.1%), while the

least number of the cases (23, 6.1%; $P=0.0001$; Table 1) were in 10< age group.

Out of 379 patients, 165 cases (43.5%) lived in the villages and 214 cases (56.5%) were urban residents ($P=0.0001$). Moreover, the majority of the patients ($n=326$, 86.0%) had a travel history, whereas 44 patients (11.6%) did not ($P=0.0001$). Moreover, with respect to nationality, 368 patients (97.1%) were Iranian and 11 cases (2.9 %) were Afghan ($P=0.0001$; Table 1).

In the current study, the patients afflicted with CL had different occupations. The highest frequency of the disease was observed in the groups of laborers and housewives ($n=61$; 16.1%), while the group associated with the least frequency were the hunters (1, 0.3%; $P=0.0001$; Figure 2).

Furthermore, the results of the study were indicative of the difference in the anatomical location of the lesions on the patients' body. The highest number of the lesions were spotted on the hands of the patients ($n=15$; 26.7%), while the lowest number was detected on the thigh ($n=14$; 2.5%; $P=0.0001$; Table 2). In addition, a number of 143 patients had only one

Table 1) Distribution of leishmaniasis patients according to demographic features of sex, age groups, location, nationality, and travel history in Mazandaran Province, 2009-2017, Number (%)

	Year								
Sex	2009	2010	2011	2012	2013	2014	2015	2016	2017
Male	14(3.7)	42(11.1)	34(9.0)	40(10.6)	35(9.2)	28(7.4)	23(6.1)	43(11.3)	20(5.3)
Female	6(1.6)	24(6.3)	13(3.4)	16(4.2)	8(2.1)	11(2.9)	12(3.2)	4(1.1)	6(1.6)
Age groups									
0-9	2(0.5)	3(0.8)	3(0.8)	2(0.5)	2(0.5)	3(0.8)	7(1.8)	0(0.0)	1(0.3)
10-19	2(0.5)	14(3.7)	6(1.6)	6(1.6)	7(1.8)	6(1.6)	1(0.3)	4(1.1)	5(1.3)
20-29	11(2.9)	29(7.7)	15(4.0)	22(5.8)	18(4.7)	14(3.7)	8(2.1)	14(3.7)	6(1.6)
30-39	4(1.1)	7(1.8)	6(1.6)	9(2.4)	4(1.1)	5(1.3)	8(2.1)	16(4.2)	7(1.8)
40-49	0(0.0)	4(1.1)	4(1.1)	8(2.1)	5(1.3)	6(1.6)	4(1.1)	8(2.1)	3(0.8)
50-59	1(0.3)	4(1.1)	7(1.8)	5(1.3)	5(1.3)	3(0.8)	4(1.1)	4(1.1)	3(0.8)
> 60	0(0.0)	5(1.3)	6(1.6)	4(1.1)	2(0.5)	2(0.5)	3(0.8)	1(0.3)	1(0.3)
Location									
City	13(3.4)	29(7.7)	28(7.4)	31(8.2)	19(5.0)	22(5.8)	22(5.8)	34(9.0)	16(4.2)
Village	7(1.8)	37(9.8)	19(5.0)	25(6.6)	24(6.3)	17(4.5)	13(3.4)	13(3.4)	10(2.6)
Nationality									
Iranian	19(5.0)	62(16.4)	47(12.4)	55(14.5)	42(11.1)	39(10.3)	31(8.2)	47(12.4)	26(6.9)
Afghan	1(0.3)	4(1.1)	0(0.0)	1(0.3)	1(0.3)	0(0.0)	4(1.1)	0(0.0)	0(0.0)
Travel history									
Yes	11(2.9)	64(16.9)	39(10.3)	50(13.2)	38(10.0)	34(9.0)	23(6.1)	43(11.3)	24(6.3)
No	2(0.5)	2(0.5)	8(2.1)	5(1.3)	4(1.1)	5(1.3)	12(3.2)	4(1.1)	2(0.52)
Unknown	7(1.8)	0(0.0)	0(0.0)	1(0.3)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

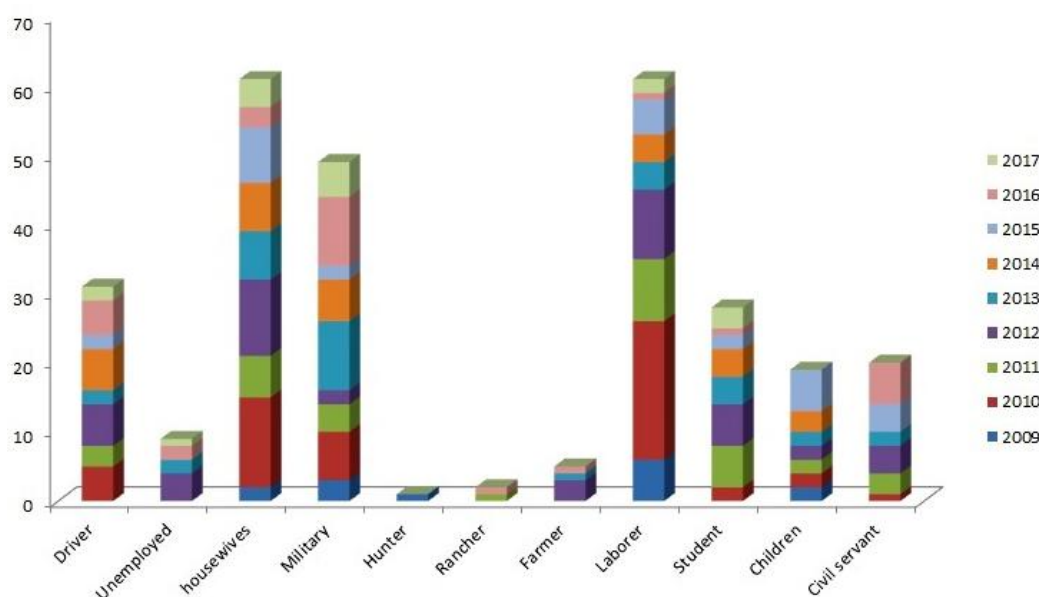


Figure 1) Distribution of leishmaniasis patients according to occupation in Mazandaran, 2009-2017

Table 2) Distribution of leishmaniasis patients according to anatomic location of lesion in Mazandaran Province, 2009-2017, Number (%)

Year	Lesion location								
	Head and neck	Trunk	Face	thigh	foot	leg	forearm	arm	hand
2009	0(0.0)	2(0.4)	5(0.9)	1(0.2)	5(0.9)	0(0.0)	4(0.7)	5(0.9)	5(0.9)
2010	3(0.5)	6(1.1)	12(2.1)	3(0.5)	26(4.6)	11(1.9)	12(2.1)	13(2.3)	24(4.2)
2011	4(0.7)	3(0.5)	4(0.7)	5(0.9)	11(1.9)	4(0.7)	12(2.1)	5(0.9)	14(2.5)
2012	2(0.4)	7(1.2)	7(1.2)	1(0.2)	17(3.0)	6(1.1)	9(1.6)	6(1.1)	29(5.1)
2013	3(0.5)	2(0.4)	2(0.4)	1(0.2)	15(2.6)	13(2.3)	8(1.4)	5(0.9)	14(2.5)
2014	4(0.7)	5(0.9)	6(1.1)	0(0.0)	11(1.9)	6(1.1)	11(1.9)	6(1.1)	19(3.3)
2015	1(0.2)	5(0.9)	7(1.2)	1(0.2)	14(2.5)	5(0.9)	8(1.4)	3(0.5)	11(1.9)
2016	2(0.4)	5(0.9)	3(0.5)	1(0.2)	17(3.0)	5(0.9)	6(1.1)	4(0.7)	20(3.5)
2017	2(0.4)	0(0.0)	2(0.4)	1(0.2)	13(2.3)	1(0.2)	2(0.4)	1(0.2)	16(2.8)

Table 3) Distribution of leishmaniasis patients according to lesion number and appearance in Mazandaran Province, 2009-2017, Number (%)

Lesion Number	Year								
	2009	2010	2011	2012	2013	2014	2015	2016	2017
1	4(1.1)	21(5.5)	18(4.7)	25(6.6)	20(5.3)	13(3.4)	12(3.2)	17(4.5)	13(3.4)
2	4(1.1)	20(5.3)	10(2.6)	9(2.4)	9(2.4)	6(1.6)	8(2.1)	14(3.7)	3(0.8)
3	3(0.8)	8(2.1)	7(1.8)	10(2.6)	3(0.8)	7(1.8)	7(1.8)	3(0.8)	4(1.1)
>3	2(0.5)	17(4.5)	9(2.4)	12(3.2)	9(2.4)	13(3.4)	8(2.1)	13(3.4)	6(1.6)
lesion appearance									
Dry	0(0.0)	28(7.4)	19(5.0)	22(5.8)	27(7.1)	20(5.3)	18(4.7)	30(7.9)	15(4.0)
Wet	0(0.0)	34(9.0)	27(7.1)	31(8.2)	13(3.4)	19(5.0)	16(4.2)	17(4.5)	11(2.9)
Lupoid	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(0.5)	0(0.0)	1(0.3)	0(0.0)	0(0.0)
not recorded	20(5.3)	4(1.1)	1(0.3)	3(0.8)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

lesion on their body, while 83, 52 and 89 patients had two, three, and more than three lesions, respectively ($P=0.0001$; Table 3).

The form of leishmaniasis was identified

using a laboratory examination of microscopic smears. *Leishmania tropica* in 109 patients (28.8%) was the most common parasites followed by *L. major* ($n=10$; 26.9%). Based on

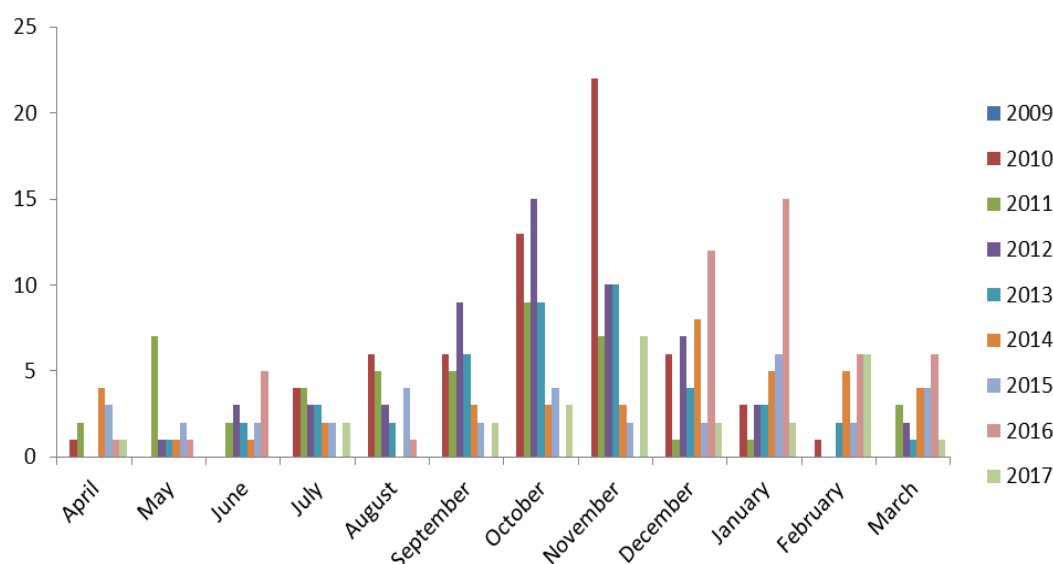


Figure 2) Distribution of leishmaniasis patients in different months in Mazandaran Province, 2009-2017

the appearance of the lesions, most of the patients ($n=179$; 47.2%) had dry lesions ($P=0.0001$; Table 3). The frequency of cutaneous leishmaniasis in Mazandaran varied in different months. The highest rate of incidence was detected in November among 61 patients (16.1%), while the lowest was related to April affecting 12 cases (3.2%) ($P=0.0001$; Figure 3).

Discussion

Cutaneous leishmaniasis has been observed in 17 out of 31 provinces of Iran (11-13). The highest disease incidence has been reported in some provinces, such as Khorasan, Esfahan, Ilam, Fars, Yazd, Bushehr, Khuzestan, whereas western and northwest provinces of the country have been associated with the lowest incidence (14). As indicated by the obtained results, the incidence rate of this disease in Mazandaran Province was estimated at 1.27/100,000 people during the study period of 2009-2017. However, the incidence rate in other regions of Iran has been reported as 76 /100,000 people in Khorasan Razavi in 2011 (14), 103/100,000 people in Aran and Bidgol, Isfahan Province in

2009 (15) and 379.1 /100,000 people in Gonbad, Golestan Province in 2010 (10). It is worthy to note that the majority of patients had a travel history; however, a travel history to endemic areas of CL was not detected in 11.6% of them. As observed in the results, the incidence rate in Mazandaran province is lower than the rest of the above-mentioned provinces. Considering that in areas with low endemicity, the history of travel is an important epidemiological factor. Therefore, some factors have turned Mazandaran Province into an endemic area for the disease. These factors entail being in the vicinity of CL endemic foci of Golestan Province and more importantly the presence of sand fly vector species in the province (16). Furthermore, owing to the fact that Mazandaran Province is the most frequently visited holiday destination, leishmaniasis needs special attention considering travelers' health

Most of the patients in this study were Iranian. In the same vein, most of the reported patients with CL were Iranian according to the studies performed in Varamin, Tehran Province (2012-2013) (1) and in Razavi Khorasan Province (2011) in Northeast of Iran (14). In addition, the majority of patients in the present study were males which was in line with the

studies carried out in Varamin (1), Kashan in central Iran (16) and in Gonbad Kavous in Northeast of the country (10). The higher leishmaniasis occurrence in men can be attributed to their greater outdoor activities and light clothing which exposes them to sandflies bits more frequently, as compared to women (10, 14).

In addition, the highest incidence rate was observed in the 20-29 age group who spend more time outdoors due to their vitality and energy. This finding is consistent with the studies carried out in Mazandaran (17), Bafgh, Yazd Province (18), Kermanshah (19), and Ilam (20) in the west of the country. The results; however, contradict those of studies conducted in Hormozgan in southern Iran (21) which reported the highest incidence in <15 age group, as well as the study performed in Kashan (22) which found the highest incidence in 0-10 years age group. It is noteworthy that when the endemicity of a certain disease is low (as in the current study), the disease occurs more in higher or all age groups alike.

Regarding patients' occupation, the highest incidence was detected in labors (16.1%) and housewives (16.1%). Nonetheless, in a previous study carried out in Khorasan Razavi, the highest rate of disease was related to the students group (14).

Furthermore, in the current study, the peak distribution of the disease was observed in autumn. The highest incidence was reported in November and the lowest in April. This finding can be ascribed to the activity season of sand flies in Mazandaran. Due to the fertility of most sandflies at the end of the sandfly season, the highest infection rate is detected at the end of the active sandfly season during October and November. This peak was reported to be in September in Damghan (23) and Gonbad Kavous (10), in Khorasan Razavi in October and November (14) and in Mazandaran in autumn (17).

As far as the anatomical location of the

lesions on the patients' body is concerned, most of the lesions were found on the hand (26.7%) which is in accordance with the studies carried out in Shiraz (24), Gonbad Kavous (25), Bafgh, of Yazd (18) and Mazandaran (17). Conversely, in another study performed in Gonbad Kavous (10), the highest percentage of lesions was observed on the feet. Nonetheless, in the studies carried out in southern Iran (26), in Marvdasht (27), as well as in Isfahan (28), the lesions were observed more frequently on the face. Leishmaniasis lesions are commonly spotted on the bare areas of the body. Therefore, the anatomical location of the lesions can vary in different parts of the world and even within the same country depending on the traditions and the manner of dressing in different areas (29).

The obtained results revealed that there was only one lesion per each patient in most of the cases (37.7%). Along the same lines, in the study carried out in Qom Province (30), most of the cases (48.89%) had one lesion; nonetheless, three or more lesions were reported in another study conducted in Ilam (20).

The results suggested *Leishmania tropica* (n=109, 28.8%) as the most common parasites followed by *L. major* (n=102, 26.9%). Species of the parasite has not been identified in some cases due to the use of traditional diagnostic methods. Therefore, owing to the lower sensitivity and specificity of traditional methods, it is recommended that molecular methods, especially Polymerase chain reaction (PCR) be used to validate diagnostic methods for a more accurate expression of the result.

Few studies have been conducted on leishmaniasis in Mazandaran and the previously performed studies have been limited in time and space. Nonetheless, the current study covers the total area of Mazandaran in a 9-year period.

Conclusion

As evidenced by the results of the current study, cutaneous leishmaniasis can be an important health issue in Mazandaran on account of several factors. These factors include agricultural activities, animal husbandry, being a holiday destination, being in the vicinity of Golestan Province which is an endemic focus of the disease. Therefore, the adaptation of surveillance and control programs should be a priority; moreover, further research on other epidemiological aspects of the disease is highly recommended in the province. In addition, in order to prevent the disease, health authorities of this province need to consider some issues, including health education, sanitary disposal of household waste, moving livestock out of villages, and prevention of the accumulation of animal manure in rural areas.

Footnotes

Acknowledgements

The current study was supported by Research Deputy of Mazandaran University of Medical Sciences, Project No. 2836. Our sincere appreciation and thanks go to the staff of health centers for their valuable contribution to this project.

Funding

The present study was supported by Mazandaran University of Medical Sciences.

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this study.

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