# The Retrospective Analysis of Cutaneous Leishmaniasis Cases in Aydin Adnan Menderes University Research and Training Hospital Parasitology Laboratory

Aydın Adnan Menderes Üniversitesi Uygulama ve Araştırma Hastanesi Parazitoloji Laboratuvarında Kutanöz Leishmaniasis Tanısı Almış Olguların Retrospektif Olarak Değerlendirilmesi

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#### Özet

Amaç: Leishmania türlerinin etken olduğu enfeksiyon hastalıklarının en yaygın formu olan kutanöz leishmaniasis (KL) ülkemizde endemik olarak görülmektedir. Çalışmamızda son altı yılda saptanan KL olgularının ve enfeksiyonla ilişkili faktörlerin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmamız Aydın Adnan Menderes Üniversitesi Uygulama ve Araştırma Hastanesi Parazitoloji Laboratuvarında Ocak 2015-Ekim 2020 tarihleri arasında KL tanısı alan olguları içermektedir. Farklı birimlerden KL şüphesiyle gönderilen olgularda direkt mikroskopi (DM) veya kültür yöntemleriyle tanı konulmuştur. Olguların cinsiyet, yaş, yerleşim yeri gibi demografik özelliklerine ek olarak, deri lezyonlarının lokalizasyonu, sayısı, süresi, tanı konulan dönem ve tanı konulma yöntemleri değerlendirilmiştir.

**Bulgular:** Son altı yılda KL saptanan toplam 79 olgunun 42'sini (%53.2) erkekler 37'sini (%46.8) kadınlar oluşturmaktadır. Olguların yaş ortalaması 37.4±23.3 olup çoğunluğu (%55.7) kırsal kesimde ikamet etmektedir. Suriye'den göç edenler olguların %15.2'sini oluşturmaktadır. Lezyonlar büyük oranda hastaların yüzünde (%63.3) yer almakta olup lezyon şikayetinin başlaması ve tanı konulması arasında geçen süre ortalama 3.6±2.7 aydır. Olguların 59'unda (%74.7) her iki tanı yöntemiyle, 16'sında (%25.3) sadece kültür, dördünde (%5) ise sadece DM ile pozitiflik görülmüştür.

Sonuç: Çalışmamız KL'nin Aydın ili için önemini koruyan bir halk sağlığı sorunu olmaya devam ettiğini ortaya koymaktadır. Ayrıca Suriye'den ülkemize göç edenlerin, hastalığın yayılmasında rolü olabileceği dikkati çekmektedir. Hastalığın laboratuvar tanısında DM'ye ek olarak kültür yönteminin de kullanılmasının gerekli olduğu düşünülmektedir.

Anahtar kelimeler: Aydın, Leishmaniasis, Kutanöz, Prevalans, Türkiye

#### Abstract

**Objective:** Cutaneous leishmaniasis (CL), the most common form of *Leishmania* infection, is endemic in our country. The study aimed to evaluate CL cases and infection-related factors in the last six years in Aydin Adnan Menderes University Research and Training Hospital Parasitology Laboratory.

Material and Methods: Our study included CL cases in Aydin Adnan Menderes University Research and Training Hospital Parasitology Laboratory from January 2015 to October 2020. The definitive diagnosis of CL was performed with direct microscopy (DM) or culture. Demographic characteristics of the cases (gender, age and residence), and localization, number, duration of skin lesions, period of diagnosis was analysed.

**Results:** In the last six years, 79 cases were diagnosed with CL, 42 (53.2%) were male, and 37 (46.8%) were female. The average age of cases was  $37.4\pm23.3$ , most (55.7%) live in rural areas, and immigrants from Syria accounted 15.2% of the cases. Most lesions were on face (63.3%) and average period from lesion and diagnosis was  $3.6\pm2.7$  months. Positive results were obtained with both methods 59 (74.7%) of the cases, 16 (25.3%) with culture method, and four (5%) with DM.

**Conclusion:** Our study reveals that CL is still an important public health problem for Aydin. In addition, immigrants from Syria may have a role in the spread of the disease. It is thought that it is necessary to use culture method in addition to DM in the laboratory diagnosis of the disease.

Keywords: Aydin, Cutaneous, Leishmaniasis, Prevalence, Turkey

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# **INTRODUCTION**

Leishmaniasis, a vector-borne parasitic infectious disease, is considered as an important public health problem in our country. Leishmania spp. are the causative agents and the disease presents three different clinical forms in humans: cutaneous (CL), muco-cutaneous (MCL) and visceral leishmaniasis (VL) (1). The most common form of the disease is CL in Turkey and two species (L. tropica and L. major) are isolated from the patients. In addition, L. infantum and L. donovani are responsible for VL cases in our country (2,3). The infection is characterized by long-term nodular ulcerative lesions and heals spontaneously within a few years, leaving unpleasant atrophic scars. It is estimated that the annual incidence is 1.5-2 million around the world and the disease is endemic in about a hundred countries mostly in tropical and subtropical regions (4). According to the World Health Organization (WHO), the majority of CL cases are reported from Iran, Syria, Afghanistan, Algeria, Brazil, Colombia, Pakistan, Peru, and Saudi Arabia. Additionally, Turkey is included among the 21 countries where the disease is endemic (5).

In routine laboratories, the diagnosis of CL is made using direct microscopy or culture methods, and the detection of amastigotes in samples from skin lesions or the enrichment of promastigotes in the medium is accepted as the gold standard (6,7). In skin lesions, "tin-tack" sign of Hulusi Behcet and ulcers (volcano shape) are defined as characteristic features for diagnosis (8). The CL is endemic in the South-eastern Anatolia; however, the prevalence is increased recently in western regions of Turkey, particularly as a result of migration. In addition, large numbers of refugees and migrants from Syria have been located in Turkey since 2011 because of the civil war. Aydin is one of the most important CL focuses following the South-eastern Anatolia region and the frequency of the disease has been increased in the last 25 years (8).

In the present study, we aimed to evaluate the frequency of CL in Aydin, Turkey for the recent six years and to study some characteristics/factors that were related to the infection.

# **MATERIALS AND METHODS**

## Study population and ethical approval

The present study included CL cases that were diagnosed in the parasitology laboratory of Aydin University Hospital from January 2015 to October 2020. The cases with suspected CL infection or lesions were sent from different clinics in the same hospital. The characteristics of individuals including age, gender, place of residence were recorded during sampling process. In addition, we observed the number, location, and duration of their lesions.

This study was approved by the Non-Invasive Clinical Research Ethics Committee of the same university (2020/62).

#### Laboratory diagnosis of CL

In the parasitology laboratory, thin smears from the lesions of cases were stained with Giemsa and amastigote form of the parasite was investigated. In addition, after injection of one milliliter (ml) of saline into the lesion, the contents of the lesion were aspirated and the fluid was inoculated in NNN medium. Following the one-week incubation at 25°C, the cultures were examined under light microscope for the presence of promastigotes. The cases were diagnosed with CL, if at least one of both methods was positive for the parasite.

#### Statistical analysis

In our study, we evaluated the sociodemographic characteristics of cases, lesion properties, and the annual and seasonal distribution of CL. Descriptive statistics were calculated using the "Statistical Package for the Social Sciences (SPSS) version 19.0" package program.

### RESULTS

In the study period, the overall number of cases diagnosed with CL was 79, the cases were sent from different clinics: the great majority (n=59, 74.7%,) was from Dermatology, 15 (19%) was from Infection Diseases, and five (6.3%) were from Plastic and Reconstructive Surgery. The highest number of cases was detected in 2015 (n=20, 25.3%), followed by 2017 (n=17, 21.5%). The number of positive CL cases by years was presented (**Figure 1**). When the distribution by months was examined, it was observed that a great majority of the cases (n=65; 82.3%) applied to the hospital with pre-diagnosis of CL between December and April (**Figure 2**).

Almost an equal distribution of CL cases was detected in terms of gender, 37 (46.8%) were female and 42 (53.2%) were male. In addition, 12 of the cases (15.2%) were Syrian refugees. The age of the cases ranged from 1 to 85 years and the average was calculated as  $37.4\pm23.3$ years. In addition, 44 (55.7%) of the cases resided in ru-

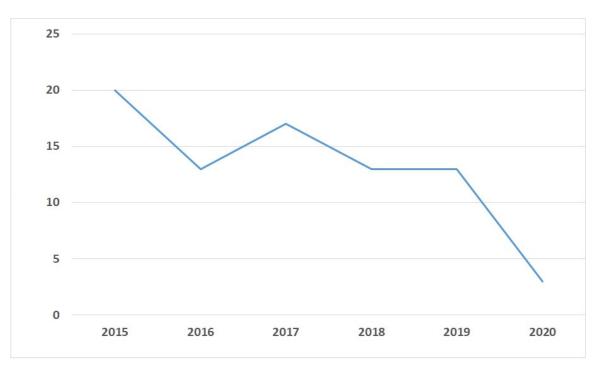


Figure 1. Distribution of CL cases by years

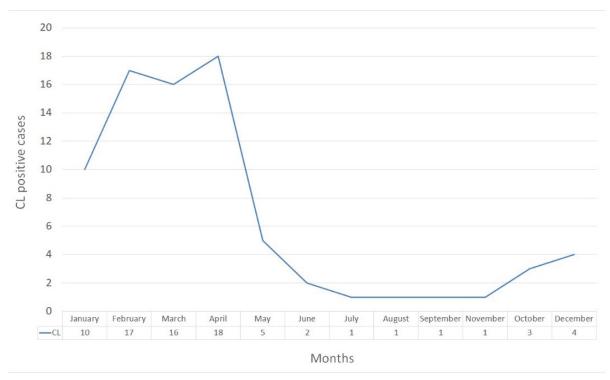


Figure 2. Distribution of CL cases by months

ral areas and 35 (44.3%) were living in urban (city or district centre).

The total number of lesions in cases varied from one to 9 with a mean of  $2.1\pm1.6$ . Lesions were observed only in the face in 40 (50.7%) of the cases and 29 (36.7%) had lesions only in extremities. In addition, lesions were observed both on the face and extremities in 10 (12.6%) of the cases. The mean lesion time was  $3.6\pm2.7$  months and 48 (60.8%) of the cases had lesions for less than th-ree months.

In most of the cases (n=59, 74.7%), positive results were detected with both diagnostic methods (**Figures 3** and 4). In addition, 16 of the cases (25.3%) were diagnosed with only the culture method, and in four (5%) only DM was positive.

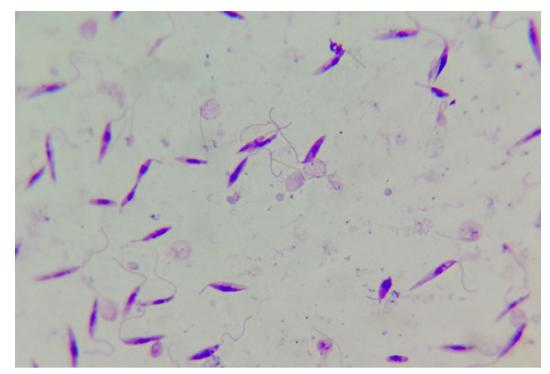


Figure 3. Leishmania spp. promastigotes in NNN medium. (Giemsa, x100)

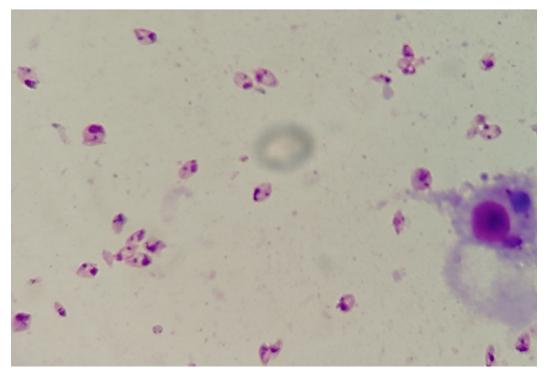


Figure 4. Leishmania spp. amastigotes in stained needle aspiration material. (Giemsa, x100)

# DISCUSSION

Turkey is among the countries where CL is endemic and the disease is considered as a public health problem (8). In our study, we analysed the CL cases for the last six years, there were 79 cases in total, and the annual average was 13.1. There were previous studies that reported CL frequency in the same city, Aydin: 73 CL cases in 1996-2000 (average 14.6); 159 cases in 1996-2004 (average 17.7), and 39 CL cases in 2012-2014 (average 19.5) (9-11). In addition, according to the records of the Health Directorate of Aydin, 45 CL cases were reported in 2001 (12). These data reported here appear to support the assumption that the number of CL cases in Aydin tends to decrease over the years. However, some factors probably limit the reliability of this assumption and should be taken into account more carefully. First, the studies generally consisted of the data from different health centres and was limited to hospital records. Secondly, different diagnostic methods were used and this caused inconsistency with the studies. For these reasons, it is thought that comprehensive field studies are required. Finally, it is important to note that the sudden decrease in 2020 (three cases) might be attributed to novel coronavirus (COVID-19) pandemic. It discouraged people to admit to the hospitals even they had lesions or complaints related to CL. Not only in positive CL cases, but also, we observed a sudden decrease in the number patients who admitted to parasitology laboratory for other parasitic diseases such as giardiasis and enterobiosis during the same period in 2020.

When the seasonal distribution of CL cases was evaluated in the present study, it was observed that the infection was most frequent in the spring and winter. As we consider the climatic conditions in Western Anatolia, it is known that the common vector of CL, *Phlebotomus* species, are especially active in April-September. Infected individuals apply to health units for diagnosis and treatment after an incubation period of 2-8 months due to varying factors. Therefore, it is observed that the cases are usually diagnosed with CL in the winter and spring months (8,10,14). In our study, in parallel with these data, most of the cases (82.3%) were diagnosed between December and April, and it was observed that the patients were admitted to the hospital after a lesion complaint for an average of 3.6 months.

In most of the cases, CL lesions were on the face, it was followed by the extremities. It was an expected finding as we consider the transmission way of the parasite and it was in accordance with many other studies (15,16). The human face is very favourable and easy to access for sand flies, and for the same reason they often suck blood from the extremities after the face. In a study from Antalya, 28 CL cases were included and it was reported that the lesions were most frequently observed in the face (64.2%) (17). In another study, this rate was reported as 67.1% (9). In our study, more than one lesion was observed in 12.6% of the cases and the mean lesion was found to be 2.1. In a study performed in Hatay, it was reported that 14.3% of the cases had more than one lesion (18). In a cross-sectional study in Sanliurfa, the average number of lesions was 1.78, while more than one lesion was observed in 19.1% of the children (19).

Environmental conditions, socio-economic status, demographic characteristics and personal habits are among the factors that affect the prevalence of leishmaniasis (20). Phlebotomus species, the well-known vector of leishmaniasis in our country, are less frequent in urban areas than in rural areas, because of the effective and intensive insecticide spraying for the control of flies. However, a similar fight in rural areas is not effective and feasible due to the presence of reservoir hosts and the wide spread of human population (21). In our study, it was observed that 55.7% of the cases resided in the village. Many studies have supported our finding and reported higher provenances in rural areas (9,22). We found that 53.2% of the positive CL cases were men. In the studies dealing with leishmaniasis and gender, it was reported that CL was more common in men, however there were studies that reported the opposite (14-16,23). In a study in Kahramanmaras, it was reported that 12 (60%) of 20 CL cases and 50.9% of them in Hatay were male (24). It was observed that 20 CL cases reported in Antalya between 2004-2006 were equally distributed between genders (25).

Among the diagnostic methods for leishmaniasis, DM is the most preferred method in most of routine laboratories. Although the method has high specificity, its sensitivity is not considered sufficient (26). When the diagnostic methods were evaluated in our study, it was seen that DM alone was insufficient, and negative results were reported in approximately one out of every four cases. In another study, 34 (92%) of 37 cases were positive with culture, while 29 (78.4%) were positive with DM (11). In a study conducted in Iran, 76.7% positivity was detected with DM, while promastigote forms were observed in only 50% of the samples with the culture method (27).

In conclusion, our study points out that CL keeps public health importance by slight changes in some years and the risk of increasing in the forthcoming years. Periodical monitoring of CL prevalence is essential to provide proper treatment facilities and control of the infection in the region. Our study also emphasized the use of two separate methods for the accurate diagnosis of the disease.

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**Author Contribution:** All authors contributed equally to the article.

**Ethical Statement:** This study was approved by the Non-Invasive Clinical Research Ethics Committee of the Aydin Adnan Menderes University (2020/62).

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