The prevalence of cutaneous leishmaniasis in Bani Walid, Libya

Short title: leishmaniasis in Bani Walid

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Abstract

Cutaneous leishmaniasis is a parasitic disease causing skin lesions on infected person. CL is endemic in Libya including Bani Walid city, where this study was carried out to find the prevalence of the disease.

Patients with cutaneous leishmaniasis attended to the health facilities, from May 2013 to October 2015, for medical help were investigated and demographic data was collected.

The ratio of infection in Bani Walid appeared to be lower than previously reported in other Libyan municipalities. The year of 2013 included the highest number of cases with the peak of leishmaniasis in the period of July to October, whereas the year of 2015 showed fewer cases. However, the number of cases differed from month to another during the same year. The ratio of infection in males is higher than in females. The number of cases varied among sub-municipalities, Tarek Al-Mattar province formed almost the quarter.

Keywords: Prevalence, Cutaneous leishmaniasis, sand fly, Bani Walid, Libya

1. Introduction

Leishmaniasis is an endemic disease in Africa, Asia, Europe, and North and South America, caused by several species of the protozoan genus *Leishmania* that lead to a spectrum of clinical diseases including cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis. *Leishmania* spp are transmitted from infected reservoir to human by infected female sand flies (Diptera: Psychodidae) when biting human for feeding on blood. They belong to two genera, *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Central and South America)(Sharma & Singh, 2008). Over 900 species and subspecies of sand flies have been identified from 88 tropical and subtropical countries (Ramalho-Ortigao

et al., 2010). Approximately, 12% of sand fly species have been confirmed as a vector of human leishmaniasis where 42% of them belong to *Phlebotomus* species in the Old World and the rest belong to *Lutzomyia* species in the New World (Maroli *et al.*, 2013). In Libya, some studies have been conducted on sand flies where 21 species of phlebotomine recorded (El-Buni *et al.*, 1993; Annajar, 1999; Elhosk *et al.*, 2014).

Cutaneous leishmaniasis is the most spreading disease that affects people health in North Africa. Thousands of cases have been reported in the countries stretching from Morocco to Libya, while a few dozen cases were reported in Egypt, from the Sinai Peninsula (Kimutai *et al.*, 2009; Aoun & Bouratbine, 2014). In Libya at least 13000 cases were reported during the early of seventies till the mid of the nineties and the transmission of cutaneous leishmaniasis has been shifted from development and agriculture projects to the villages. The most important agent for the leishmaniasis in Libya is the *L. major* MON-25 (Annajar, 1999), followed by *L. tropica* and *L. infantum* (Amro *et al.*, 2012; Belal *et al.*, 2012).

Cutaneous leishmaniasis is endemic in Libya, occurred in many areas including the Al Jabal Al Gharbi and Bani Walid (Aoun *et al.*, 2006; Belal *et al.*, 2012; Abdellatif *et al.*, 2013; Ahmed & Aboufaddan, 2013). According to the National Centre for Disease Control (NCDC) in Libya for the period from 2010 till 2012, 1584 cutaneous leishmaniasis cases were reported (NCDC, 2013). Cutaneous leishmaniasis affects all age groups with slight higher ratio in males than females (Amro *et al.*, 2012). In Bani Walid, about 120 cases were reported during the same period. However, these figures may not include all the infected people in the city, as figures were recorded during and after the armed conflict, thus many cases could have been missed out. Therefore, this study was conducted to shed light on the effect of cutaneous leishmaniasis on health and focuses on its prevalence in humans in Bani Walid.

2. Material and methods

2.1 Study site

This study was conducted in Bani Walid city, which is located in the north-western Libya (13°58'59"E and 31°59'00"N), about 180 km south east of Tripoli (Figure 2.1). The area of the city is 19,710 km^2 and the total population of Bani Walid is 98,424 according to the registry office survey in 2013.

2.2 Sample collection and geographic distribution

Patients with cutaneous leishmaniasis lesions attended at Bani Walid medical clinics and Bani Walid general hospital during the period from 12-05-2013 to 31- 10-2015 were included in this study. The cutaneous leishmaniasis cases were clinically diagnosed and confirmed by microscopic examination. The following epidemiological data were collected from each patient: age, gender, place of residence, number and location of lesions, and the date of lesion appear. A consent was obtained from each adult patient or the responsible person for the children cases.

2.3 Microscopic examination

The examination of the lesion to confirm the infection was done following the standard method described (Amro *et al.*, 2012). As follow:

Firstly, the skin was cleaned and sterilized with disinfectant. Then biopsies were taken from the lesions by sterilized and disposable scalpel blade for making smears on microscope slides. The Wright's Giemsa stain was used to stain the smears then examined by light microscopy searching for amastigotes. Microscope magnification was 400 X. Positive slides were stored by medical clinics in Bani Walid for further studies.

2.4 Statistical analysis

The obtained data were statistically analyzed using non-parametric tests, considering $P \leq 0.05$ as significant. All statistical analyses were carried out on SPSS 20.0 statistical software (SPSS, 2011).

3. Results

A total of 218 cutaneous leishmaniasis cases were diagnosed, during the period from May 2013 to October 2015 in the Bani Walid district which harbors around 98400 inhabitants. Accordingly, the average of the annual incidence was estimated to be 0.097% (between 0.055% and 0.178%).

The cases belonged to certain sub-municipalities of Bani Walid (Tarek Al-Mattar, Ad-Dahra, Al-Lawteen, Al-Manasla, Al-HaiAsenaai, Al-Souk, Eshmikh, and Tassneea). The cases of Tarek Al-Mattar formed almost the quarter with 54 cases followed by Ad-Dahra48 cases (22.01%), Al-Lawteen 36 cases (16.51%); Al-Manasla31 cases (14.22%), Al-Hai Asenaai 23 cases (10.55%) and Al-Souk 21 cases (9.63%): Eshmikh and Tassneea showed few cases (Table 3.1).

Males formed 64.74% of the cases (141 cases) while females formed 35.3% (77 cases) which differed significantly (χ^2, $P< 0.0001$).

The age cases ranged between 2 and 75 years (median age was 29 years, with an average age 28.47±14.66 (Table 3.2). The mean age of males was 28.65±13.84, whereas the mean age of females was 28.13±16.05. There were no significant differences between the mean ages with respect to the gender ($U=4666$, $P= 0.76$). The majority of the cases (93.6%) were between 6 and 50 years of age, with the highest percentage 24.31% occurring in the 15-23 years age group.

The number of cases (Table 3.1) differed significantly among years (χ^2, P< 0.0001) except between 2014 and 2015 (χ^2, P=0.55). However, almost the half of cases was recorded in the period between June and November 2013 (50.9%) as shown in figure 3.1.

The number of lesions per case ranged from one to four. 144 patients (66.06%) had one lesion and 74 patients had multiple lesions (Table 3.3). The majority of lesions were located on the exposed parts of the body i.e. face, hands and the rest were on the other parts of the upper and lower limbs.

4. Discussion

Cutaneous leishmaniasis is a major public health problem and endemic in many countries all over the world, such as; Iraq, Kuwait, Iran, Afghanistan, and in the Middle East and North African states (Belal *et al.*, 2012; Rahi *et al.*, 2014). Cutaneous leishmaniasis is the most spread disease affecting human health in North Africa countries including Libya, where the infection is considered an endemic (Kimutai *et al.*, 2009; Aoun & Bouratbine, 2014). The main leishmania parasite form responsible for the disease in Libya is *Leishmania major* MON-25 (Annajar, 1999; Aoun *et al.*, 2006; Amro *et al.*, 2012; Abdellatif *et al.*, 2013; Ahmed & Aboufaddan, 2013; Aoun & Bouratbine, 2014). The presence of infection in Libya was dated back since 1910 (Annajar, 1999); then thousands of cases have been recorded from different cities in the country especially from the west part including Bani Walid city, which is one of the Libyan endemic areas (Kadiki & Ashraf, 1971; Annajar, 1999; Dokhan, 2008). The number of cases in Bani Walid is differing from sub-municipalities to another depending on the population size and infrastructure development. According to the NCDC, the number of cases in between and 2012 passed 120 cases (NCDC, 2013). This study has recorded the number of patients with leishmaniasis lesions that sought medical treatment or diagnosis in the city health facilities. Compared to other studies that were conducted in Libya, the results

showed that the percentage of the annual incidence was less than what have been recorded in other cities such Sirte and Nalut (Fathy *et al.*, 2009; Belal *et al.*, 2012).

The results showed that there is a significant difference between leishmaniasis infection in males and females. This finding is similar to other reports from Libya (El-Buni *et al.*, 2000; Shoaib *et al.*, 2007; Fathy *et al.*, 2009; Belal *et al.*, 2012; Abdellatif *et al.*, 2013). This could be related to the outdoor activities of men (working duties and leisure). The infected age groups in this study were ranged between 6 and 74 years with the highest percentage occurring in the 15-23 years age group. El-Buni and Ben-Darif (1996) reported that the most affected age group in Al-Badarna, Libya was 1-10 years; In Nalut, Libya, the youngest people formed almost 80% of cutaneous leishmaniasis cases (Belal *et al.*, 2012); in another study in Al-Jabal Al-Gharbi, the most affected age group was between 11 and 35 years old (Abdellatif *et al.*, 2013). The reason for low rate of elderly patients may be related to the fact that they were infected during early ages and they acquired long term immunity during childhood (Zaph *et al.*, 2004). Another factor is that older people do not admit to a health centre for the treatment of cutaneous leishmaniasis while they know this disease and disfiguring scars are not as important for them as for youngsters. Additionally, the outdoor activities of the young people are more than the other age groups in which, youngsters are exposed to the disease more often (Akçalı *et al.*, 2007).

The sub-municipalities of Tarek Al-Mattar, Ad-Dahra and Al-Lawteen showed to be the most infected sub-municipalities. This can be related to that Tarek Al-Mattar is a newly developed area and many have new built houses and farms, whereas Al-Dahra sub-province is the most populated area in Bani Walid. Al-Lawteen is a very large area with a large number of inhabitants. The most likely reason is an increase in human- sand fly contact, which is attributed to the development of villages and the spread of the human population into the habitats of the local vectors (Rahi *et al.*, 2014). Eshmikh and Tassneea sub-municipalities

showed few cases and this may be due to the far distance from the health facilities that are located in the city centre and the population is relatively small.

The period of June to November showed to have most cases, but the number of cases differed between years; in previous studies in Libya, the most prevalent infection was during the November– February period (El-Buni *et al.*, 2000; Fathy *et al.*, 2009; Belal *et al.*, 2012). Surprisingly, in the year 2014 there were no cases during the period of January to March. The incubation period of the parasite takes from 4 to 12 weeks after the bite of the sand fly (Aransay *et al.*, 2000). Amro *et al*. (2012) noticed that the cases of cutaneous leishmaniasis caused by *L. major* occurred mainly in the period between November and January, while the highest peak of cutaneous leishmaniasis caused by

L. tropica was in February.

Many patients had multiple lesions on the exposed parts of the body and moreover, the prevalence of multiple lesions is significantly higher among adult patients than single lesions, and the same pattern was reported previously (Abdellatif *et al.*, 2013). Some cases were found to be infected for the second time with leishmaniasis, this supports the presence of more than one species of leishmania in Bani Walid as has been reported by Aoun *et al*. (2006), that *Leishmania killicki* was identified in Libya from Bani Walid. *Leishmania major*, *L. tropica*, *L. infantum* and *L. donovani* are the causative agents of cutaneous leishmaniasis in Libya (Amro *et al.*, 2012; Belal *et al.*, 2012). In Libya, diagnosis and treatment of cutaneous leishmaniasis patients are provided free of charge by the state government, however, many infected people in Bani Walid avoid admission to any medical centre since they have got used to the disease and its complications for many years. Therefore, we assume that the impact of the disease and its incidence has been underestimated. More studies are required about the foci of Leishmania and the sand fly species responsible for transmission of

infection in Bani Walid to obtain the full picture of the disease and establishing a good control programme.

Conclusion

Cutaneous leishmaniasis is an endemic in Bani Walid with hundreds of cases were reported in the last decade, however annual incidence during the present study period was low in comparison with other cities. Therefore, we assume that the impact of the disease and its incidence has been underestimated, and people need to be educated about the importance of seeking treatment of the disease. The results in this study perform the foundation to encourage more studies to cover all the aspects of the disease in Bani Walid and to study the reservoir host and the vector sand fly to obtain the full picture of the disease and to establish a good control programme.

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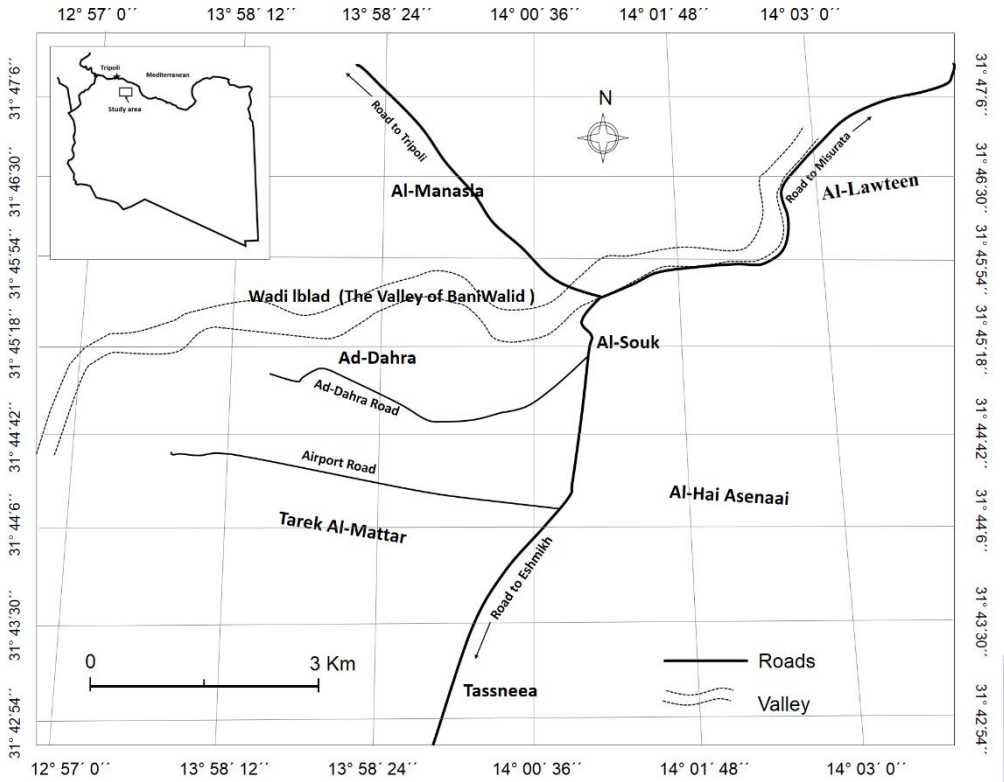

Figure 2.1. The map of study area showing the sub-municipalities of Bani Walid

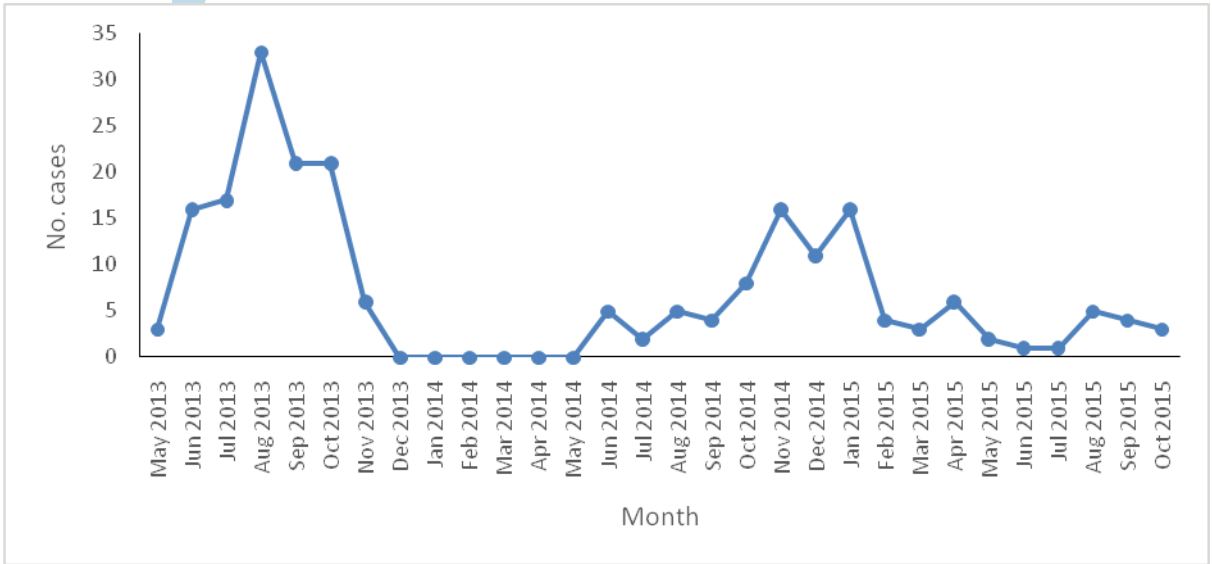

Figure 3.1 The number of reported cutaneous leishmaniasis in Bani Walid between May 2013 and October 2015.

Table 3.1. The number of cutaneous leishmaniasis cases among different sub municipalities of Bani Walid, Libya.

Sub-municipalities	2013	2014	2015	Total
Tarek Al-Mattar	37 (31.62%)	9 (16.07%)	8 (17.78%)	54 (24.77%)
Ad-Dahra	22 (18.8%)	17 (30.36%)	9 (20.01%)	48 (22.01%)
Al-Lawteen	10 (8.55%)	16 (28.57%)	10 (22.22%)	36 (16.51%)
Al-Manasla	24 (20.51%)	5 (8.93%)	2 (4.44%)	31 (14.22%)
Al-HaiAsenaai	17 (14.54%)	2 (3.57%)	4 (8.89%)	23 (10.55%)
Al-Souk	5 (4.27%)	6 (10.71%)	10 (22.22%)	21 (9.63%)
Eshmikh	2 (1.71%)	1 (1.79%)	1 (2.22%)	4 (1.84%)
Tassneea	0 (0.0%)	0 (0.0%)	1 (2.22%)	1 (0.47%)
Total	117 (100%)	56 (100%)	45 (100%)	**218 (100%)**

Table 3.2. Age groups and gender of cutaneous leishmaniasis cases in Bani Walid

Age group (years)	Male	Female	All
6>	8 (5.67%)	3 (3.89%)	11 (5.05%)
6-14	13 (9.21%).	13 (16.88%)	26 (11.93%)
15-23	34 (24.11%)	19 (24.68%)	53 (24.31%)
24-32	38 (26.95%)	7 (9.09%)	45 (20.64%)
33-41	20 (14.19%)	19 (24.68%)	39 (17.89%)
42-50	19 (13.48%)	11 (14.29%)	30 (13.76%)
51-59	6 (4.26%)	2 (2.60%)	8 (3.67%)
59>	3 (2.13%)	3 (3.89%)	6 (2.75%)
Total	141 (100%)	77 (100%)	218 (100%)

Table 3.3. Number of lesions per cutaneous leishmaniasis patient in Bani Walid.

No of lesions	Number of cases (%)			
	2013	2014	2015	All
1 lesion	64 (54.7%)	41 (73.2%)	39 (86.67%)	144 (66.06%)
2 lesions	36 (30.8%)	13 (23.2%)	5 (11.11)	54 (24.77%)
≥ 3 lesions	17 (14.5%)	2 (3.6%)	1 (2.22%)	20 (9.17%)
Total	117 (100%)	56 (100%)	45 (100%)	218 (100%)