

ANALYSIS OF CLINICAL FEATURES WITH MICROSCOPY OF CUTANEOUS LEISHMANIASIS SUSPECTED PATIENTS ATTENDING THE DERMATOLOGY CLINIC AT GENERAL HOSPITAL HAMBANTHOTA.

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INTRODUCTION

Leishmaniasis is a vector borne parasitic disease which affects 12 million people globally and estimated two million new cases occurring every year (WHO 2009). Rather than a single disease entity leishmaniasis has a wide spectrum of disease manifestations resulting mainly in 3 clinical forms. Visceral leishmaniasis (VL), Mucocutaneous leishmaniasis (MCL) and Cutaneous leishmaniasis (CL). Among all three forms, CL is most frequent in Sri Lanka.

Leishmaniasis was declared as notifiable tropical disease in Sri Lanka in year 2008. Up to date there were more than 2000 CL cases reported and it is now an established disease (Siriwardena *et al* 2003, Nawarathne *et al.*2007) affecting almost all the provinces in this country. Even though not potentially fatal or debilitating as VL or MCL forms, CL can be personally and socially disruptive (Herwaldt 1999) due to different types of clinical presentations which results disfiguring scars.

Vectors of leishmaniasis are sandflies belonging to a subspecies of the genus phlebotomous. *Leishmania donovani* the causative organism of CL is transmitted between sandfly vector and the human host, the main host for this species. However, recently it was found that domestic dogs had shown the presence of *Leishmania* parasites, providing primary evidence of an animal reservoir in Sri Lanka (Rosypal *et al* 2010).

On taking blood meal from host, infected female sandfly regurgitate the flagellar *leishmania* promastigotes into the skin, which invade or are phagocytosed by host cells primarily macrophages causing the typical cutaneous lesion. The disease starts with a macule then a papule, which enlarges and then becomes an ulcer, with a rare possibility of the lesions remaining non-ulcerative and diffused. They often end up as skin lesions with a raised edge and a central crater (Fact sheet Epidemiology unit 2012). The clinical presentation of CL in Sri Lanka, are non tender non itchy papules, scaling nodules or ulcers affecting mainly exposed areas in the body specially limbs and face. (Siriwardena *et al* 2003, Nawarathne *et al* 2007) Lesions may heal spontaneously within weeks to months, or last for a year or more. Although there were several clinical manifestations, the morphologic appearance of lesions is known to vary depending on the species or strain of the causative organism (Dedet *et al* 1989) and the immune status of the patient (Guessous-Idrissi *et al* 1997).

Diagnosis of cutaneous leishmaniasis is based on clinical symptoms, microscopic observation of parasites in stained tissue smears, culture of promastigotes or by molecular detection methods such as PCR. Microscopic identification of amastigotes in stained preparations varies depending on the number of parasites present and /or the experience of the person examining the slide (Magil *et al* 2005).

As there were no studies described to evaluate parasite loads using the slit skin smear in different clinical presentations this study aimed to describe the correlation of *L.donovani* amastigote parasite load in Giemsa stained smears with CL clinical presentation patterns in Sri Lanka.

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METHODOLOGY

Sampling

Skin samples were taken from suspected CL lesions of 87 patients referred to the Dermatology clinic, General Hospital, Hambanthota. Clinical presentations of the lesions were examined and photographs were obtained with the consent of the patient. Lesions and the adjacent normal looking skin around them were cleaned and sterilized with soap, normal saline, and 70% ethanol consequently. For making stained smears tissue was taken using a disposable scalpel blade. Skin tissue was smeared on a clean glass microscopic slide, fixed with 100% methanol for one minute, and stained with 10% Giemsa. The stained tissue smears were examined for the presence of characteristic amastigotes by light microscopy using Olympus microscope at 100x oil immersion magnification. The number of amastigotes was counted by two individuals as double blind study using WHO described method.

Parasite load calculation

The amastigote numbers of skin smears were graded according to WHO recommended grading (WHO 2010) as follows;

- 6+: > 100 parasites per field
- 5+: 10–100 parasites per field
- 4+: 1–10 parasites per field
- 3+: 1–10 parasites per 10 fields
- 2+: 1–10 parasites per 100 fields
- 1+: 1–10 parasites per 1000 fields
- 0: 0 parasites per 1000 fields

Clinical feature assessment

Clinical categorization as papule, nodule, nodulo-ulcerative, ulcer (dry/wet) and plaque was made by the consultant dermatologist according to previously described method (Ranawake *et al* 2012, Bari *et al* 2006).

Ethics Statement

The study was approved by and carried out under the guidelines of the Ethical Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura. All patients provided written informed consent for the collection of samples and subsequent analysis.

RESULTS AND DISCUSSION

Different types of clinical presentations were observed and characterized (Figure1). Out of the specimens from 87 suspected CL patients 30 Dry ulcers, 8 nodular ulcerative lesions, 28 nodules, 4 papules 8 plaques, 2 satellite lesions and 7 wet ulcers were noted. 49/87 lesions were positive for SSS. Most frequent clinical presentations were nodules and dry ulcers (66%) (Table1). The duration of lesions varied from 1- 36 months. Majority of the lesions were within 1-6 months (83.9%).The sites of the lesions varied from most commonly in the arm , leg, forearm, face, ear ,neck, abdomen, and chest.

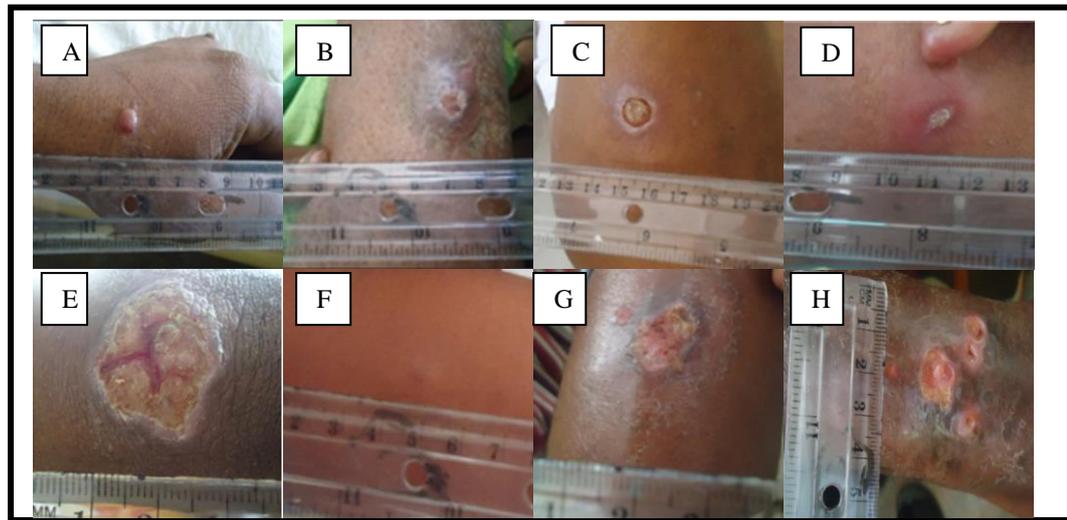


Figure 1. Spectrum of clinical presentations

A. Nodule B. Ulcerative nodule C. Dry Ulcer D. Plaque E. Wet ulcer F. Papule G, H. Satellite lesions

The parasite counts seen in the SSS of the patients in relation to the clinical presentations of the lesion are given below (Table 1).

Table 1. Parasite counts in SSS in relation to clinical presentation (n=49)

Parasite count	Dry ulcer (N=22)	Ulcerative nodule(N=5)	Nodule N=(15)	Papule N=(2)	Plaque N=(3)	Satellite lesion N=(1)	Wet ulcer N=(1)
1+	4	3	2	-	-	-	1
2+	10	1	5	2	1	-	-
3+	5	1	4	-	1	1	-
4+	1	-	1	-	1	-	-
5+	2	-	2	-	-	-	-
6+	-	-	1	-	-	-	-

Out of the 49 SSS positive lesions, 84% showed relatively low parasite counts ranging from (1+) to (3+). The (3+) gradings mainly included dry ulcers (n=5/22) and nodules (n=4/15).

A wide range of parasite burden was observed in dermal tissues of CL patients with nodules and dry ulcers showed more parasite loads in comparison with other type of lesions. It has been reported in previous studies, that *Leishmania* parasites are scanty and difficult to demonstrate in skin lesions with papular presentation (Salotra *et al* 2003). Our study confirmed these observations. However, only small number of papular lesions was found in our study. This may be due to ignorance of patients to present to treatment, as papules are the

earliest clinical manifestation of CL and due to the fact that they are single, non-tender, non-itching and usually dry (Ranawake *et al* 2012). Individuals seek treatment when, the lesion which had started as a papule and then gradually enlarged and ulcerated, with changes in the surrounding skin. The clinical presentation results having nodules and ulcers as the highest form of presentation supports this theory. Parasite numbers may increase when the papules enlarge to form nodules and ulcers when they remain untreated. Our results showing 3+ parasite counts with nodules and ulcers may be due to this reason.

Direct microscopy with slit smears of CL lesions is reported to have low sensitivity (13–60%) (Zijlstra *et al* 2000). Similar observations were made in slit smears of CL patients with 30% positivity in patients with nodular lesions, 44% positivity with dry ulcers, and 4% positivity with papules. The duration of the lesions of most nodules and Dry ulcers were between 1- 12 months. In a study by Siriwardana *et al* 2008 showed that nodules with 5-9 months duration had the highest parasite positivity.

CONCLUSIONS/RECOMMENDATIONS

In this study parasitic load was overall low in all lesions. This may be due to the fact that *L.donovani* Mon -37 strain in Sri Lanka is naturally attenuated (Mccall *et al* 2013). Also it was shown that only 49 out of 87 CL suspected patients were parasitologically positive for Cutaneous leishmaniasis. Absence of such suspected lesions may be due to clinical manifestations of leishmaniasis which could imitate several other conditions such as insect bites, tropical ulcers, leprosy etc. Results of this study indicate that perhaps, in the absence of an animal reservoir, nodules and ulcers that are rich in LD bodies could be the main human parasite source for transmission in the community.

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