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EPIDEMIOLOGICAL FEATURES AND CLINICAL MANIFESTATIONS OF CUTANEOUS LEISHMANIASIS: AN EMERGING HEALTH RISK IN HAMBANTOTA, SRI LANKA

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ABSTRACT: Cutaneous leishmaniasis (CL) is an established disease in Sri Lanka. Present study was designed to investigate the epidemiological features, clinical manifestations and the relationship between CL cases and rainfall in Hambantota district, Sri Lanka from March, 2014 to December, 2015. CL suspected patients who presented to Tangalle and Hambantota hospitals during this period were included in the study. After conducting a clinical examination, a descriptive study was carried out using a questionnaire. Slit-skin smears were taken, stained with Giemsa and examined under a light microscope to identify *Leishmania* amastigotes. Results showed that the highest CL cases were reported from Tangalle District Secretory Division (DSD) out of 12 DSDs in Hambantota district ($P<0.05$). Majority of patients were $\geq$50 years old. Males were more infected than females. Higher number of CL patients were students (x^2 (10) = 6.41, P=0.781). Most of the patients had single lesion on arms, legs and faces/cheeks. Size of the most lesions was >2mm -<1cm and the duration of the most lesions were <06 months. The common clinical characteristics of lesions were erythematous and dry. Further, findings revealed that the higher number of CL cases were reported in March, May, August, October in 2014 and January to March in 2015 in which the study area had received higher amount of rainfall. Findings of the present study showed that the CL incidences and its distribution within Hambantota district had increased. It is essential to consider CL as a public health problem in Hambantota district.

Key words: Cutaneous leishmaniasis, *Leishmania* sp. lesions, clinical features, Hambantota

INTRODUCTION

Leishmaniasis is endemic in more than 90 countries including India, Bangladesh, Nepal and Pakistan. It is a protozoan parasitic disease caused by

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Leishmania species and about 12 million people are infected worldwide (WHO, 2020). Leishmaniasis has three clinical forms according to the location of parasites in mammalian tissue, namely cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL). CL is the most prevalent type of leishmaniasis and it has two clinical forms such as diffuse CL and local CL (Desjeux, 1996). For decades, in Sri Lanka, leishmaniasis was considered as an exotic disease until local transmission of leishmaniasis was first evident from Southern part of Sri Lanka in 1992 (Athukorale *et al.* 1992; Siriwardena *et al.* 2003). In 2003, localized cutaneous leishmaniasis was reported from north-central province also (Siriwardena *et al.*2003). Until 2011, clustering cases of CL had been reported in a few areas mainly in Hambantota and Polonnaruwa districts in Sri Lanka. A few cases of visceral leishmaniasis were reported from Anuradhapura district in 2005 and 2007 (Abeygunasekara *et al.*2007). In 2011, 940 cases of CL were reported in Sri Lanka out of which 723 cases were confirmed through field investigations (Karunaweera *et al.*2020). The causative *Leishmania* spp. of CL in Sri Lanka is *Leishmania donovani* MON 37, the most virulent species (Karunaweera *et al.* 2003) and it is usually the causative organism of visceral leishmaniasis in the Indian subcontinent (Siriwardena 2008).

In old world, CL infection originates from the bite of sand flies of the genus *Phlebotomus.* In Sri Lanka, two species of *Phlebotomus* are reported; *P. argentipes* and *P. stantoni*. *P. stantoni* is a jungle species feeding on wild rodents. *P. argentipes* is also anthropophagic (Leishmaniasis Fact sheet 2012). Both zoophilic and anthropophilic behavior patterns of *Phlebotomus* had been identified (Lewis and Kendrick1973, Surendran *et al.* 2005, Nawaratna *et al.* 2009, Karunaweera *et al.* 2009, Gajapathy *et al.* 2011). *P. argentipes* could be putative local vector of leishmaniasis in Sri Lanka. Mammals such as canids and rodents act as reservoirs of *L. donovani* in many CL endemic countries (Nawaratna *et al.* 2009).

Currently, CL is reported from many parts of Sri Lanka including southern region and it has become an established disease in the country.CL is a disfiguring disease with the potential long term psychological and social impacts on infected persons. The target area of this study had been Hambantota district which belongs to Southern province of Sri Lanka. Even though a considerable amount of CL patients had been reported from Hambantota district in 2004 (Rajapaksa *et al.* 2007), the current status of the disease in this area has been changing. *Leishmania* species of Sri Lanka may have co-evolved with vectors and potential reservoir hosts and the ecological conditions of the country (Kariyawasam *et al.* 2017). Environmental conditions such as humidity, atmospheric temperature, land degradation, global warming and rainfall

influence the distribution of parasites (Bari and Rahman 2008). However, Bhattarai *et al.* (2010), Ready (2014) and Akter *et al.* (2016) suggested that *L. donovani* is strictly anthroponotic. The aim of the present study was to examine the epidemiological features, clinical manifestations and periodicity of CL cases in the Hambantota district, Sri Lanka after about ten years. That will be a prerequisite to formulate an appropriate control method and to predict the risk of CL for the community of the study area.

MATERIAL AND METHODS

The study was conducted from March, 2014 to December, 2015 in Hambantota district located in the coastal belt of Southern part of Sri Lanka (Coordinates are 6.2467°N, 81.0755°E). Its northern and western margins are boarded by the Sabaragamuwa province and the Western province respectively. The total land area is 2609 km^2 and it consists of 12 District Secretory Divisions (DSD)(figure1). The total population is 599,903 and population density is 229.9 inhabitants/km^2 (Sri Lanka Census of population and housing 2012). The monthly average rainfall data of the Hambantota district during the study period was obtained from the records of the meteorological department of Sri Lanka (Climate Data.Org/, Hambantota 2021).

Patients included in this study were those who presented themselves to the Tangalle Base Hospital and Hambantota general Hospital during the period from March, 2014 to December, 2015. After a complete clinical examination was performed on each patient by the clinician, a retrospective descriptive study was carried out. The study objectives were clearly informed verbally participants by the investigator and written consent was taken from each participant and the guardians of children. Information of participants and lesion samples were always kept confidentially. CL suspected patients were interviewed through clinical history information such as size, number, type, site and duration of lesions (months) and clinical features such as dry/wet, pain, itchy, erythematous, presence of hypo-pigmented halo and swelling/ enlargement of lymph nodes and presence of satellite nodules were recorded. The details of demographic parameters such as age, gender, residential area and occupation were recorded by the investigator.

Diagnosis of CL cases was done by using slit-skin smear preparation. On the first day of enrolment to the hospital, with the guidance of dermatologists of both hospitals, slit-skin smears were taken from each suspected patient. Slit-skin smears were taken at the most indurated margin of the lesion under aseptic conditions. Prepared thin smears were air-dried and fixed with methanol. CL patients were identified by examining Giemsa stained slit- skin

lesion smears microscopically and *Leishmania* amastigotes were observed in slit-skin smears of CL patients (Plate 1). In this profile, CL patients were reported from all DSDs in Hambantota district except Lunugamvehera DSD (Fig.1). After the smear was air-dried again, it was examined under a light microscope with oil immersion for the presence of *Leishmania* amastigotes. Leis*hmania* amastigotes were identified having ovoid/round shape body and characterized by a distinctive nucleus and nearby small kinetoplast. Each smear was examined two times to improve the accuracy of the test. Ethical approval for this study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka. (Ref. No.: 26.06.2014:3.1).

A database was generated with Excel using individuals as statistical units. SPSS 25.0 (SPSS Inc, Chicago IL, USA) software was applied to perform descriptive statistics on geographical distribution of CL patients and demographic data. *Leishmania* smear positivity was calculated as the number of smear positive subjects (CL patients) among the study group. Chi-Square tests (Pearson) were accomplished to determine the significant differences in geographical distribution, sex, age-groups and occupations of patients at $P<0.05$ or $P<0.001$ level. Clinical manifestations of CL lesions were described using summary statistics. Monthly distribution of CL cases and monthly average rainfall in the study area were presented graphically for the period from March, 2014 to December, 2015.

RESULTS AND DISCUSSION

Cutaneous leishmaniasis is an established protozoan parasitic disease in Sri Lanka, especially in north central province and southern province. Twenty-six CL cases were reported from Beliatta DSD in Hambantota district during the period of 2003-2004 (Siriwardena *et al.* 2003, Rajapaksa *et al.* 2007, Nawaratne *et al.* 2009). A total number of 420 CL patients were enrolled in both Tangalle Base hospital and Hambantota General Hospital during this study period from March, 2014 to December, 2015. The highest number of CL cases was from Tangalle DSD (n=209) and Beliatta DSD (n=123) was the second. Ambalantota, Katuwana, Walasmulla, Weeraketiya, Okewela, Angunakolapelessa, Hambantota and Sooriyawewa, DSDs also had 45, 3, 3, 11, 8, 7, 8 and 2 CL patients respectively. Further, Tissamaharama DSD had only one patient. Distribution of CL cases among DSDs in Hambantota district was statistically significant (x^2 (10) = 63.255, $P<0.05$). The highest number of CL cases was from Tangalle DSD which is highly populated, urban area. Further, Tangalle DSD is neighboring to Dickwella DSD which is the highest CL prevalent area of Matara district (Kariyawasam *et al.* 2015). Tangalle, Beliatta and Ambalantota DSDs are located in the Southern border of the district and adjacent to each other (Fig.1). Further,

a considerable amount of CL cases was found from Weeraketiya, Okewela, Angunakolapelessa, Sooriyawewa and Hambantota DSDs also. Our finding was supported by the findings of Jayathilake and Taylor-Robinson (2020) and Karunaweera *et al.* (2021) which indicate the increased transmission intensity and spreading of the disease from coastal area to inland areas of the southern hotspots. Yet, no any CL patient was reported to both hospitals from Lunugamvehera DSD. Sometimes, CL patients of Lunugamwehera may get treatments from the Moneragala general hospital which is closer than the Tangalle or Hambantota hospitals

Age of the studied patients was ranged from 1 to 78 years. Majority of the participants (33%) belong to the age group $\geq$ 50 years old and the lowest number of patients (n=56) was within 20-29 years age group. However, there was no statistical significant difference in CL cases among different age groups (x^2 (4) = 3.145, P=0.534) (Fig. 2). Rajapaksa *et al.* (2007) reported that the most infected age group was younger generation (10-19 years) in southern part of Sri Lanka in 2004. Siriwardena *et al.* (2003) and Nawaratne *et al.* (2009) also indicated that the highest incidence rate of disease was detected among age group of 31-40 years old in Sri Lanka. In contrast, most of the CL patients belonged to age group of 1-10 years old in Genaveh in Iran (Kasari *et al.*2014) and majority of patients under 24 years of age in Pakistan where CL is a rising epidemic (Khan *et al.*2016). By considering all these findings, we can assume that intensity of CL in various age groups depend on the intensity of the illness endemicity and immunity level and occupation/ outdoor activities of infected patients.

Distribution of gender based CL cases in the study area was also analyzed. In the current study, the male /female ratio was > 1 and it was observed that a higher number of males affected (58.6%) than females (41.4%). This difference was not statistically significant (U= 20333.0, P=0.383) (Fig.2). Nawaratne *et al.* (2009) indicated that the same pattern such as the number of CL infected males was higher than that of females. In the initial phase of CL outbreak of the country, more males in 21-40 year age-group affected and the majority of them were soldiers working in northern part of the country (Siriwardena *et al.* 2003). As a result of more outdoor activities, males may possibly expose to outdoor sand-fly vectors than the females. Another, study done in Matara district, which is adjacent to Hambantota district, indicated that the majority of infected patients were between 21 and 50 years (Kariyawasm *et al.* 2015).

Occupations of the studied CL patients were considered and it was noted that the highest percentage of CL patients was students (20.5%) (School children and university students). Outdoor activities of students such as participating in schools and extra classes, leisure and recreation activities during early mornings and also in dusks may increase their chance of being bitten by sand-fly vectors.

In contrast, Siriwardena *et al.* (2003) reported that the majority of affected cases in Sri Lanka were male soldiers. The second largest group of CL patients in the current study was represented by housewives. Similarly, Rostami *et al.* (2013) also indicated that most CL infected patients in Central Iran were housewives. As an occupation category, all housekeepers are women while all men, in the present study were divided into various income generating occupations. Other occupational categories included in the study were farmers (7.4%), laborers (1.2%), fishermen (3.8%), skilled-workers (5.5%), government servants (7.9%), others group (includes retired and private sector patients) and military personal (0.7%). A few (6.3%) CL patients had no any occupation. There was no statistically significant difference in CL cases among different occupations (x^2 (10) = 6.41, P=0.781). Considerable percentage of self-employed persons such as three-wheel and bus drivers, brick makers and small businessmen were among the CL patients in the present study. However, a few retired and private-sector workers, government servants, farmers, skilled workers, fishermen, laborers and military personal were also found.

The clinical manifestations of CL lesions were studied and it was observed that all localized lesions were seen on exposed body sites of CL patients. Totally 235 patients had lesions in their arms followed by legs (n=165) and face/cheek (n=64) respectively (Plate 2). Presence of CL lesions on arms and legs in patients in Sri Lanka was also reported by Siriwardena *et al.* (2003). Similar observations were reported by Rostami *et al.* (2013) in Central Iran and Kassiri *et al.* (2014) in Genaveh country. In contrast, Nawaratne *et al.* (2007) and Rajapaksa *et al.* (2007) reported that most of the lesions were found in faces and upper limbs of patients. Four types of lesions such as nodules, papule, ulcers and plaque were found in this clinical profile and majority of lesions were nodules, followed by ulcers (Table1). Siriwardena *et al.* (2003) also had observed three types of lesions such as single or multiple dry ulcers, single scaling nodules and erythematous papules in their investigation. Yet, Nawaratne *et al.* (2007) categorized lesions according to their appearance. However, morphological appearance of lesions is varied 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). Over a half of the CL patients in the present study had one skin lesion and a few number of patients had two lesions. Similarly, most of the CL patients from other areas of the country also had single lesions (Navaratne *et al.* 2007 and Rajapaksa *et al* .2007). In the present study, a few patients had multiple lesions (more than three lesions). Sand flies usually have a discontinuous blood-sucking habit and may sting several times at every attack and cause the development of

several lesions on the skin. The sizes of CL lesions in the current profile, varied from 1mm to larger than 3cm. Majority of lesions were between 2mm to 1cm

Table 1: Clinical features of lesions of CL patients in the present study

Features	Frequency (n)	Percentage (%)
Site of lesion		
Face/Cheek	64	11.8
Ear	11	2.0
Arm	235	43.7
Leg	165	30.7
Belly	14	2.6
Neck	09	1.7
Backside	30	5.6
Other (lip, shoulder, fingers)	1 0	1.9
Type of lesion:		
Nodules	284	48.5
Papule	105	17.9
Ulcers	184	31.4
Plaque	13	2.2
Number of lesions:		
One	347	82.6
Two	52	12.4
Three	14	3.3
Four	2	0.5
Five	1	0.2
More than five	4	1.0
Lesion size:		
<1mm	186	32.4
>1mm-<1cm	203	35.4
>1cm- <2cm	124	21.6
>2cm-<3cm	47	8.2
> 3cm	14	2.4
Duration of lesions (months):		
< 6	345	69.7
>6-<12	141	26.2
> 12	22	4.1
Lesion characteristics:		
Dry	192	35.7
Wet	23	4.3
Erythematous	136	25.3
Hypo pigmented halo	46	8.5
Pain/Itchy	9	1.7

Swelling/enlargement of lymph nodes	4	0.7
Satellite nodules	2	0.4

(Table 1). Twelve patients had lesions larger than 3cm and all of them were ulcers with secondary infections.

The duration between the first detection of lesion and initial presentation for treatments was ranged from one week to 36 months and 345 (69.7%) lesions were less than six months old. Twenty-two (4.1%) lesions were greater than 12 months (Table1). Siriwardena *et al.* (2003) reported that the duration of CL

lesions varied between 2 months to 6 years and Nawaratne *et al*. (2007) also indicated that CL patients had lesions ranging in duration from 2 weeks to 4 years. That implies current CL patients keen to get treatment earlier. Findings of the current study showed that lesions had different characteristics. One hundred and ninety-two lesions were dried meanwhile twenty-three lesions were wet. Large number of lesions were erythematous and 46 lesions were with hypopigmented halo. Enlargement of lymph nodes and satellite nodules were observed in a few number of lesions (Table1). Supportively, Siriwardena *et al*. (2003) also reported about the satellite nodules around the main lesion. The lesions on most patients were dry and scaly, but on some, they were wet and a few patients showed a hypopigmented halo around the lesions (Navaratne *et al*. 2007). Thus, the clinical manifestations of CL may vary according to the causative organism. Akilov *et al*. (2007) reported that the general absence of itchy and pain are characteristic features of CL lesions. Similar results were found in the current study but a few number of lesions had pain/ itchy. It was interesting to find out that among 420 CL patients, only 10 patients had an early history of infection of CL in this study profile (Table 1). Reyburn *et al*. (2003) reported that about 21% had a history of CL in Kabul which was considered as an endemic area for anthroponotic cutaneous leishmaniasis in Afghanistan.

Vector species and their behavior are important factors that influence the prevalence of CL in humans (Grimaldi and Tesh; 1993). Lewis and Killick-Kendrick (1973) reported that two species of *Phlebotomus* sand flies had been identified from Sri Lanka (*P.argentipes* and *P. sergenti*). Presence of *P.argentipes* had been reported for many years in Sri Lanka and it is the well-known vector of *L. donovani* which is the causative parasite of visceral leishmaniasis in India (Lane *et al.*1990). After Lewis and Killick-Kendrick (1973), the presence of *P. sergenti* had not been reported so far from Sri Lanka (Surendran *et al*. 2007). Still there is lack of knowledge on vector species of CL in Sri Lanka. However, there may be a close association between the increase in numbers of CL cases and amount of rainfall received the study area. Hambantota district is considered as a dry lowland area. The mean temperature in Hambantota district is 27.2^{0}C and it varies by 1.8^{0}C. It gets large amount of rainfall during two monsoonal rainy periods from November to January and May to June. The city gets 1,257 millimeters (49.4 inches) of precipitation annually. Average temperatures in Hambantota change little throughout the year, ranging from 26.3 °C (79.3 °F) in January to 28.1 °C (82.6 °F) in April and May (Climate Data.Org/, Hambantota 2021). According to the findings of the current study, the average number of CL patients presenting was 24 for a given month during the study period. During two monsoon seasons, Hambantota district receives

high of rainfall. So, high rainfall, high humidity and low temperature in the area may be optimal to sand flies for their breeding, resulting population increase in sand flies. Higher number of human exposure to the vector sand flies in this

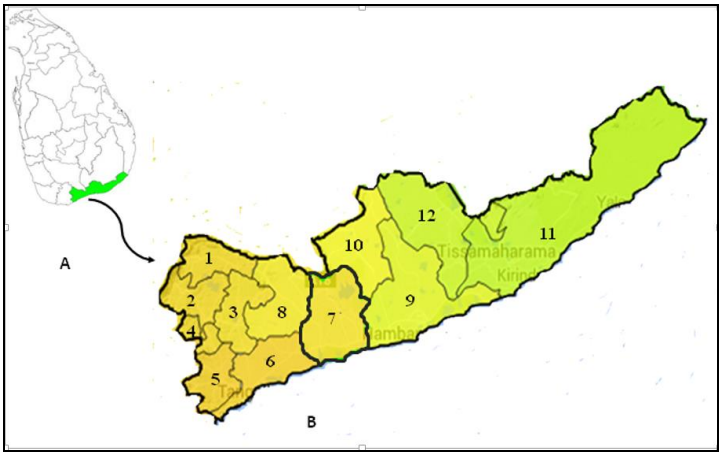

Fig. 1: A: Map of Sri Lanka with Hambantota district shaded. B. District Secretary Divisions. DSDs are: 1. Katuwana, 2. Walasmulla, 3. Weeraketiya, 4. Okewela, 5. Beliatta, 6. Tangalle, 7. Ambalantota, 8. Angunakolapelessa, 9: Hambantota, 10: Sooriyawewa, 11: Tissamaharamaya, 12. Lunugamwehera (Source: Adapted from Google maps)

area may increase the number of CL cases. Hence, monthly distribution of CL cases during this study period in study sites of Hambantota district was analyzed to find out whether there is any relationship between CL cases and rainfall in this area and results are presented in figures 3 and 4. The highest number of CL cases (n=45) was reported in October, 2014 (Fig. 3). According to figure 4, the study area received large amount of rainfall during the periods from May to June and from October, 2014 to January, 2015. Further, current findings indicated that number of CL cases had increased in May, August, October in 2014 and January, February in 2015 in which the study area had received higher of rainfall than the other months. On the contrary, very high rainfall may flood sand fly breeding places and wash off the organic matter and destroy the immature stages of sand flies such as larvae and pupae. This may decrease the size of sand fly populations, results in decreasing the human exposure to sand flies and low number of CL cases in the area.

Results of present study also proved that Hambantota district received the highest rainfall in December, 2014 during which the fewest number of CL cases was reported. Rutledge and Elllenwood (1975c) also indicated that the survival and/or development of immature sand flies in Neotropical areas depends on the accumulation of organic matter and protection from flooding, rainfall, direct light and wind and occasionally, very severe or very mild weather may also cause unusual troughs or peaks in a sand fly population (Chaniotis *et al.*1971).

In dry months, lack of plant growth and rain decrease the sand fly population densities and fewer incidence of vector-human contact and hence, fewer number of CL cases in the study area. Yet, Galgamuwa *et al.* (2018) reported that there was a negative correlation between monthly average rainfall and leishmaniasis cases in Hambantota district during the period from 2009-2016.

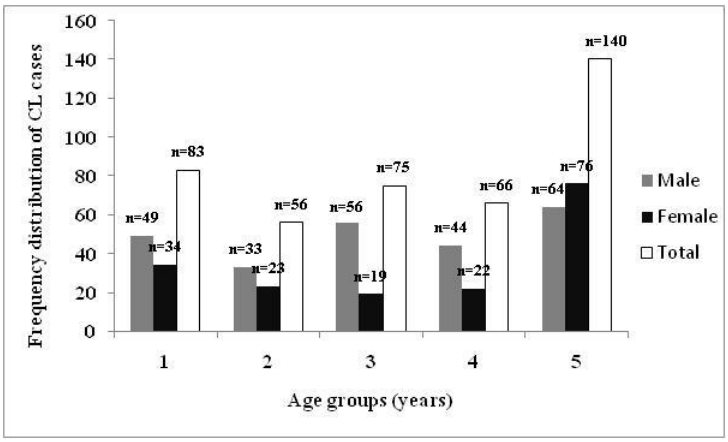

Fig. 2: Age and gender based distribution of CL cases in the study sites of Hambantota district during the study period. Age groups were: **1**: ≤ 19yrs., **2**: 20-29 yrs., **3**: 30-39 yrs., **4**:40-49 yrs., **5:** ≥50 yrs.

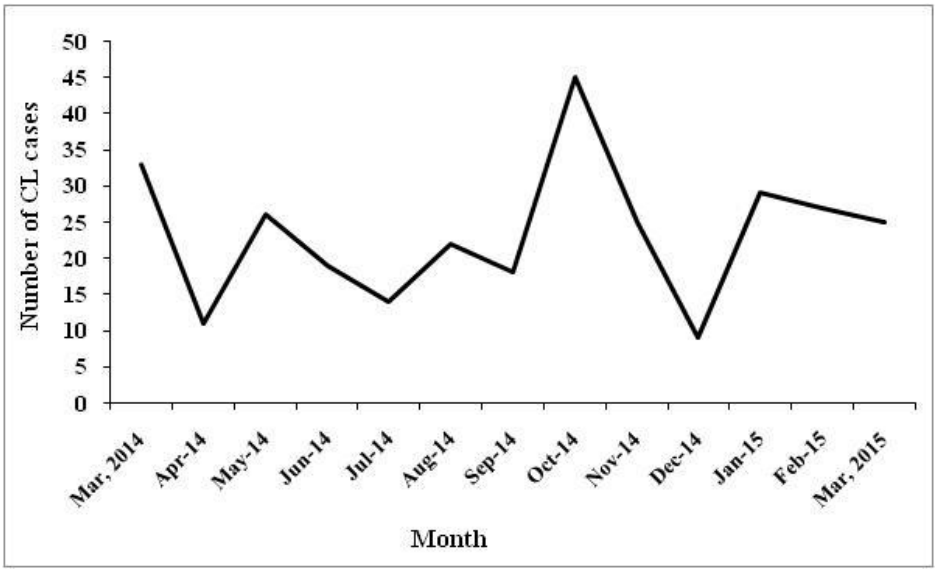

Fig. 3: Monthly distribution of CL cases during the study period in Hambantota district.

Even in rainy months, the study area had received the amount of rainfall less than 350mm which may not be enough to flood in the study area. However, more and long term studies are needed to explain the ecological factors which

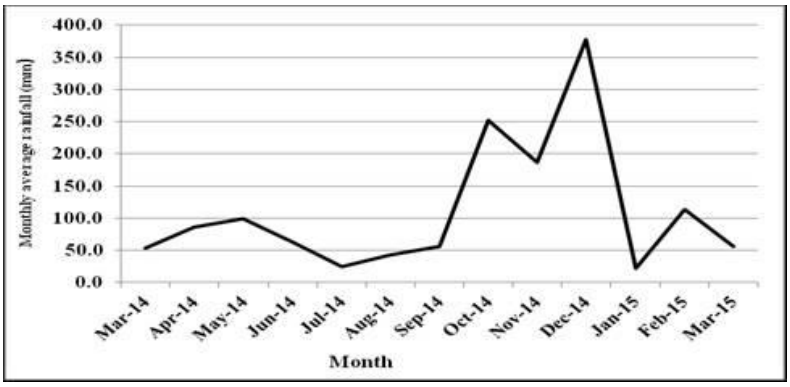

Fig. 4: Monthly average rainfall in millimmeter in Hambantota district during this study period.

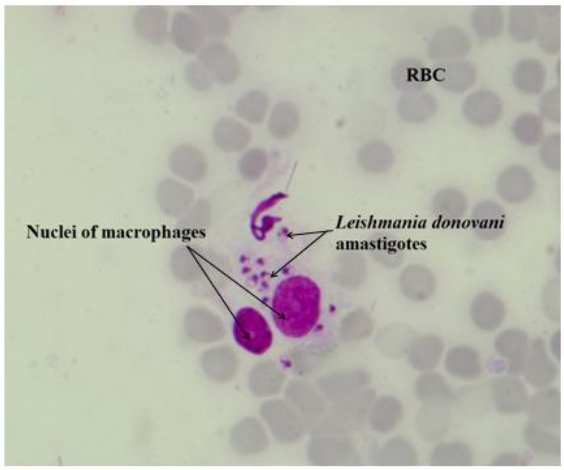

Plate 1: *Leishmania donovani* amastigotes, are seen inside two macrophages in a Giemsa stained thin smear of a CL patient in Hambantota district (X1000), **RBC**: Red blood cells.

Plate 2: A: CL lesions (papule type) on a leg, B: CL lesions on nose and lips of CL infected patient in Hambantota district (black arrows indicate lesions).

help to increase the CL cases in this study area as infections were found to be dependent on abundance of the vector sand flies, type of cutaneous leishmaniasis and prevalence of reservoir hosts.

CONCLUSIONS

The present study indicates that the distribution of CL cases in Hambantota district had changed and CL infected patients were found in new adjacent DSDs during ten years period after 2004. Number of CL cases had also increased from March, 2014 to December, 2015. Most of the CL patients were more than 50 years old and large number of CL patients were house-wives and students who may be infected due to their outdoor activities at sandfly feeding and mating hours such as in dawn and dusk. Majority of the patients had one lesion on exposed body parts. Monthly distribution of CL cases was more coincided with the rainfall pattern of the study area during the study period. More studies on possible vector populations and reservoirs and their role in transmission of parasite are essential to identify in Hambantota district.

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