DOI: 10.1111/pde.14195

Pediatric Dermatology WILEY

Clinical, microscopic and molecular presentation in pediatric versus adult old world cutaneous Leishmaniasis

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Abstract

Background: Cutaneous leishmaniasis (CL) is an emerging uncontrolled tropical parasitic disease in endemic and nonendemic areas with a high prevalence in the pediatric age group.

Method: A total of 382 individuals from Lebanon, Saudi Arabia, Pakistan, and Syria diagnosed with CL by punch biopsy/scrapings were grouped into adults (>18 years) and pediatrics (≤18 years). Data recorded included clinical features [number, location, type, size, and extensiveness (size larger than 3 cm, more than 5 lesions per patient, lesion present for more than 12 months, special types, disfiguring lesion or closeness to vital sensory organs) of lesions] and microscopic findings [Ridley's Parasitic Index and Ridley's Pattern]. In addition, molecular confirmation and speciation were performed.

Results: In comparison with adults, patients in the pediatric group (n = 158, 41.4%) showed significantly higher number of lesions, more facial involvement, and more extensive disease ($P < .05$). Microscopically, a more advanced Ridley's pattern was observed. The other variables did not show statistical difference between the two groups.

Conclusion: Historically, CL has been known to be a neglected tropical disease of poverty and pediatric predilection. In our pediatric group, CL manifests with more extensive disease clinically mirrored by more advanced lesions microscopically.

KEYWORDS
inflammatory disorder, insect bite

1 | INTRODUCTION

Leishmaniasis is a parasitic disease transmitted via the bite of the sand fly vector Phlebotomus.[1] It is endemic in more than 90 countries with the number of new cases per year ranging between 700 000 and 1 200 000 according to WHO,[2] and is most prevalent in the Middle East and North Africa. However, with increased international travel among populations and changes in the vector's habitat, there is a marked increase in the incidence of this disease.[3] It can be classified largely into three forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, and visceral leishmaniasis.[4] The natural history of CL starts with a papule 1 week following the sand fly bite that progress to a nodule/plaque which ulcerates within 5-7 months ending with a scar in 8-12 months. For this reason, "old world leishmaniasis" is also called an annual sore.[5] While not fatal, CL can lead to significant morbidity as well as social stigma. CL can arise at any age, often causes cosmetic disfiguration[6] and can compromise the function of vital sensory organs[7] (eyes, ears, nose, and mouth) due to secondary infection and closeness of lesions to such organs.[8]

Leishmaniasis is one of the so-called neglected tropical diseases.[6] Its disease burden and epidemiology are poorly surveyed,

and treatment of the disease needs further study and development.[9] Leishmaniasis mainly affects populations living in poverty, without adequate sanitation and in close contact with infectious vectors and domestic animals and livestock.[10] CL typically appears on exposed body sites.[11] No vaccines or drugs to prevent infection are currently available. The best way for travelers to prevent infection is to protect themselves from sand fly bites.[12] The pediatric population has been known to be highly affected by CL,[6,13] with a risk equivalent to or even higher than the adult population.[13,14]

The aim of this study is to compare childhood and adult CL. Epidemiological, clinical, and microscopic findings in addition to molecular speciation were assessed and compared between both groups in a large multiregional "Middle East" cohort.

2 | MATERIALS AND METHODS

2.1 | Case selection and clinical data

A search of the pathology and dermatology archives at the American University of Beirut Medical Center, Beirut, Lebanon; Tishreen University, Latakia, Syria; Saad Specialist Hospital, Al Khobar, Kingdom of Saudi Arabia; and Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan was performed. The search included patients with skin lesions diagnosed with CL between 1992 and 2016. Three hundred and eighty-two patients with CL from Lebanon, Syria, Saudi Arabia, and Pakistan were evaluated. Part of this material has been used in a previous publication by the same group.[1,5,15] Clinical data collected included the patient's age, gender, geographic location, eruption type (papular, nodular, verrucous, or scar), duration, and anatomic site. Extensiveness, a category adopted by the WHO after several publications described its importance on disease progression and treatment, was also documented.[16] CL is considered extensive when at least one of the following criteria is fulfilled: disease compromising the function of vital sensory organs (eye, ear, nose, and mouth); lesions >3 cm in diameter; more than 5 lesions; disfiguring facial lesions; special forms, such as sporotrichoid or lymphangitic with satellite lesions; and lesions present for >12 months duration.

Treatment was performed according to the WHO guidelines,[16] patients who did not have extensive lesions underwent weekly meglumine antimonate (glucantime) intralesional injections until initial cure. Patients who had extensive disease had weekly meglumine antimonate intramuscular injections until initial cure. Initial cure is defined by scarring and disappearance of inflammatory lesions.

2.2 | Microscopic evaluation and sample preparation

Formalin-fixed paraffin-embedded tissue blocks of 4.0-mm punch biopsies were obtained for each patient. The biopsies were stored in tightly sealed formalin-filled tubes. Formalin-fixed paraffin-embedded (FFPE) blocks of the biopsies were prepared in the standard manner[17] Tangential scrapings were performed with a scalpel and transferred to glass slides at room temperature.[18] All cases were reviewed by an expert pathologist (IK). The modified Ridley's Parasitic Index (PI) (Table 1) and Ridley's Pattern (RP) (Table 2) were recorded.[19] RP was not performed on the patients who were diagnosed by scrapings only. The PI was grouped into 3 categories: low PI (scores 0-1), intermediate PI (scores 2-3), and high PI (scores 4-6). The RP was also grouped into early (1-3) or advanced (4-5).

2.3 | Molecular analysis

All included cases were sent for PCR confirmation with subsequent molecular analysis performed according to our previously reported protocol. In brief, for FFPE tissue blocks, DNA was extracted from ribbons originating from FFPE. For scraping, the DNA extraction was done using the Pel-Freez (Invitrogen, Carlsbad, CA) kit according to the standard manufacturer's instructions. The DNA samples were incubated at 65°C for 30 minutes and then stored at 4°C until analyzed by PCR. The resultant DNA was then quantified using the Biomate spectrophotometer (Thermo, Glasgow, UK).[17]

2.4 | Inclusion/exclusion criteria

Untreated patients who had a confirmed diagnosis by PCR and had available complete clinical data were included in this study. Cases with visceral or mucocutaneous involvement, insufficient material for PCR or microscopic examination were excluded from the study.

TABLE 1 Modified Ridley's Parasitic Index

Parasitic index	Number of amastigotes per standard section
6+	≥100 000
5+	≥10 000
4+	≥1000
3+	≥100
2+	≥10
1+	≥1

TABLE 2 Ridley's Pattern

Group	Description
I	Almost normal; patches of collagen degeneration
II	Predominant severe necrotizing process in the dermis
III	Diffuse and heavy mixed inflammatory infiltrate in dermis
IV	Scattered Langerhans giant cells and primitive epithelioid histiocytes
V	Well-formed granulomas and well-developed epithelioid histiocytes

TABLE 3 Comparison of Adult and Pediatric Groups with Cutaneous Leishmaniais

		Adult	Pediatric	ρ
Number of patients		224 (58.6%)	158 (41.4%)	
Gender	Male	118 (52.7%)	92 (58.2%)	0.28
	Female	106 (47.3%)	66 (41.8%)	
Anatomic location	HN*	86 (38.4%)	94 (59.9%)	0.0001
	UE*	73 (32.6%)	21 (13.3%)	
	LE*	47 (21.0%)	20 (12.7%)	
	T	6 (2.7%)	5 (3.2%)	
	HN and UE	6 (2.7%)	5 (3.2%)	
	HN and LE	0 (0.0%)	3 (1.9%)	
	UE and LE	6 (2.7%)	2 (1.3%)	
	HN, LE, and UE	0 (0%)	8 (5.1%)	
Lesion type	Papule	11 (4.9%)	4 (2.5%)	0.69
	Plaque and nodule	115 (51.3%)	81 (51.3%)	
	Ulcer and Verrucous	95 (42.4%)	71 (44.9%)	
	Scar	3 (1.3%)	2 (1.3%)	
Average lesion duration (months)		7.77 ± 0.79	6.53 ± 0.53	0.23
Average number of lesion*		2.01 ± 0.13	2.69 ± 0.25	0.01
Extensive disease*		70 (31.3%)	84 (53.2%)	0.0001
Categorical Parasitic Index	Low	108 (48.4%)	69 (45.7%)	0.78
	Intermediate	33 (14.8%)	21 (13.9%)	
	High	82 (36.8%)	61 (40.4%)	
Ridely's pattern	Early*	116 (53.0%)	56 (39.7%)	0.01
	Advanced*	103 (47.0%)	85 (60.3%)	
Molecular	L. Tropica	202 (90.2%)	147 (93.0%)	0.43
	L. Major	22 (9.8%)	11 (7.0%)	

Abberivations: HN, head and neck; LE, lower extremity; T, trunk; UE, upper extremity.
*$P < .05$

2.5 | Statistical analysis

Continuous variables were analyzed by t test rank-sum test as appropriate. Categorical variables were analyzed using chi-squared test. A two-tailed *P* value of <0.05 was required for statistical significance. Analyses were performed using SPSS version 23 (IBM Inc, Somers, NY, USA).

3 | RESULTS

3.1 | Patient demographic, microscopic, and molecular findings

Patients' age ranged from 1 to 92 years (mean 27.6 ± 21); 158 patients (41.4%) were less than18 years of age. No significant gender predilection was noted. Most patients were examined in Lebanon (n = 214), followed by Pakistan (n = 71), Syria (n = 54), and finally Saudi Arabia (n = 43). The number of lesions ranged from 1 to 23 lesions per patient (mean 2.3 ± 2.6). The duration of the lesions at time of diagnosis ranged from 9 days to 132 months (mean 7.26 ± 9.94 months). Clinically, most patients had advanced lesions at presentation characterized by ulceration (43.5%) or plaques (51.3%). Microscopically, amastigotes were not detected in a large number of patients (n = 147, 39.3%). However, more than half of the patients had advanced RP (4-5) (n = 188, 52.2%). The majority of patients had *Leishmania tropica* as the culprit diagnosed by molecular speciation (n = 348; 91.1%). 82% of patients had initial cure after standard treatment with meglumine antimonate.

3.2 | Adult versus Pediatric involvement with CL patient analysis

In our database, 41.4% of patients were in the pediatric group (Table 3). Pediatric patients had higher number of cutaneous lesions per patient, more involvement of the head and neck region, more extensive disease and more advanced RP ($P < .01$) when compared

to adult patients. Other variables showed no significant statistical difference.

3.3 | Pediatric involvement with CL patient analysis

In the pediatric group, the average age was 9.0 ± 0.8 years. The gender ratio between male and female was 1.4 (Figure 1). When comparing male patients to female patients, no significant statistical difference was noted in anatomic location of lesions, number of lesions, PI, RP, or molecular speciation. The same was noted when comparing the pediatric population stratified according to age (older than 10 years versus younger than 10 years). Both head and neck involvement (odds ratio 1.8) and pediatric age group (odds ratio 2.1) were found to be independently predictive of extensive disease using a multivariate analysis.

4 | DISCUSSION

Cutaneous leishmaniasis is increasing throughout the world due to many factors including deforestation, human migration, urbanization and to a lesser extent malnutrition. These factors are affecting both the host's immunity and the vector's (sand fly) habitat.[3,20] This uncontrolled tropical parasitic disease found in both endemic and nonendemic areas is highly prevalent in pediatric patients.

In the literature, pediatric patients comprised between 30.2% and 46.6% of reported cases.[6,14,21-23] Our study showed similar incidence of CL to what has been reported in the literature with 41.4% of patients younger than 18 years. Male preponderance was identified (58.2%) in our cohort. This is similar to several other studies (54.1%-63.7%).[13,14,21,23] However, other studies reported a female preponderance.[22,24]

Previous studies noted the number of lesions per patient in pediatric CL ranged between 1.1 and 1.7.[13,23-25] Single lesions were noted in 51.3%-100% of pediatric patients.[6,14,22,26] In addition, Zaraa et al found no significant difference between the percentage of patients with single or multiple lesions when comparing adult and pediatric groups.[6] Although our study showed a similar incidence of pediatric patients with single lesions (56.3%), a higher average number of multiple lesions (2.7 ± 3.2) was noted. Furthermore, a statistically significant difference was observed when comparing the average number of lesions between pediatric and adult groups (2.0 ± 2.0). Aksoy et al proposed that the greater number of lesions in children may be due to the lack of immunity in children since they lack previous exposure to the disease.[24]

In addition to the clinical analysis, our study also examined microscopic and molecular speciation of all subjects which was not previously reported in the literature. In our series, the pediatric patients showed significantly more advanced RP, with no significant difference in PI. A low PI was seen in 45.7% of patients, which highlighted the instrumental role of PCR in diagnosis and speciation. This advanced RP in the pediatric patients can be either due to the delayed presentation or more aggressive nature of the disease in children due to the immature immune system.[24]

When comparing the pediatric group with the adult group, several studies have documented a higher involvement of the head and neck region in the pediatric group. Zaraa et al found that 88.1% of the lesions in the pediatric CL were in the head and neck region versus 63% in the adult group.[6] Al-Tawfiq et al even documented a gender difference in anatomical distribution in the pediatric population.[27] In our study, 59.9% of pediatric patients had only head and neck lesions versus 38.4% in the adult population.

As we documented in a previous study,[2] the pediatric group also was found to have significantly more extensive disease. A similar finding was also noted in this larger cohort, and patients with head and neck involvement had a lower average age and more extensive disease compared to patients with no head and neck involvement.[2] Furthermore, extensiveness and lower average age were found to be significant independent factors when correlated with head and neck

FIGURE 1 Pediatric cutaneous leishmaniasis

involvement using a binomial multivariate analysis. These findings can be probably attributed to the prolonged period children spend sleeping and the parts of the body exposed at the time of sleep.[13,25]

Throughout the literature, several treatment options are available including azoles[28,29] which have a modest efficacy and a reasonable side-effect profile.[28] Our treatment protocol with meglumine antimonate, in keeping with current WHO guidelines,[16] provided an 82% initial cure rate which is similar to the literature.[30]

This study's limitations include the exclusion of patients with incomplete charts and the retrospective nature of the study which may create selection bias.

In this large multiregional cohort, different clinical, microscopic, and molecular aspects in CL were assessed in pediatric patients. It also elaborates the differences between pediatric and adult groups. The pediatric group had higher number of lesions, more head and neck involvement, more extensive disease, and more advanced lesions.

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How to cite this article: Dunya G, Loya A, Taraif S, Adib Houreih M, Khalifeh I. Clinical, microscopic and molecular presentation in pediatric versus adult old world cutaneous Leishmaniasis. *Pediatr Dermatol*. 2020;37:656–660. https://doi.org/10.1111/pde.14195