Parasitology

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Epidemiological and pathological characteristics of Cutaneous Leishmaniasis from Baluchistan Province of Pakistan

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Research Article

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Abstract

Cutaneous Leishmaniasis (CL) is considered a neglected tropical disease which in Pakistan can now be considered as a growing public health problem. The exact figures on the magnitude of the disease are lacking both at the national and regional level and only a few health centres are available for diagnosis of CL. The present study was designed to identify the epidemiology of CL infection from August 2018 to December 2019 and to assess clinical aspects of CL in Baluchistan Province of Pakistan. A total of 4072 clinically suspected CL cases were analysed statistically. The highest number of CL cases were reported in May, followed by April, January and then July, February and June and the lowest number of cases were observed in March and November. The highest prevalence rate was found in males where 38% of reported cases were aged 0–9 years. The majority (24.4%) of lesions were found on the hands followed by the face in which cheeks, ears and nose were the effected organs. About 50% of the participants have single lesion while 14% of the participants had two and nearly 3% of the participants have six lesions. The atypical clinical presentations were observed in Baluchistan and common unusual presentations were lupus erythematosus. The study findings suggest that more epidemiological studies and health education campaigns are needed for the population awareness regarding CL in Baluchistan. It is recommended that risk factors should be evaluated to establish control and management strategies to prevent disease at the individual and community level.

Introduction

Leishmaniasis is a group of parasitic infections caused by flagellate protozoans of genus Leishmania that are transmitted through the bite of infected female phlebotomine sand fly (Elmahallawy *et al*., 2014). More than 20 species of Leishmania are capable of causing the disease (Georgiadou *et al*., 2015; WHO, 2020) with clinical manifestations including Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis, Diffuse Cutaneous Leishmaniasis and Visceral Leishmaniasis (also known as kala-azar) (Desjeux, 2004; WHO, 2010; Ghatee *et al*., 2020). Leishmaniasis has been reported in 98 countries affecting about 350 million people worldwide, making it one of the seven most common as well important neglected tropical diseases (Torres-Guerrero *et al*., 2017; de Souza *et al*., 2018). CL being the most common and widely distributed variety claims between 0.7 and 1.2 million cases reported worldwide annually (Alvar *et al*., 2012). About 95% of the incidents are reported from three epidemiological regions i.e. South America, the Mediterranean Basin and spread throughout the Middle East to Central Asia (Torres-Guerrero *et al*., 2017). More than 85% of new CL cases appeared in Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iran, Iraq, Pakistan, the Syrian Arab Republic and Tunisia during 2018 (Zijlstra, 2016; WHO, 2020).

CL is highly endemic in Pakistan and spread extensively due to massive out-migration from endemic to non-endemic areas and vice versa (Kassi *et al*., 2008). CL affects people of low social-economic status and is associated with undernutrition, population displacement, lack of shelter and a weak immune system (WHO, 2010). The burden of disease in Pakistan has been reported to be surpassed by 400 000 cases reported in 2016 that makes about 10% of CL cases globally (Blum *et al*., 2004) with both anthroponotic (ACL) and zoonotic (ZCL) forms of CL (Alvar *et al*., 2012). ACL occasionally occurring due to *Leishmania tropica,* is widespread and most grave public health problem in Pakistan (Hussain *et al*., 2018). The urban areas of Punjab and Sindh provinces are of high CL endemicity (Iftikhar *et al*., 2003; Kakarsulemankhel, 2004; Katakura, 2009), the Afghan refugee camps

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Table 1. Description of the study sites

Study site	Coordinates	Altitude (m)	Total HUs	Climate				
				Annual average rainfall (mm)	Annual Average humidity (%)	Temperature (°C)		
						T min	T max	T Average
Chagai	28.98° N, 62.45° E	850	29 746	47	34	6	37	22
Quetta	30.17° N, 66.97° E	1679	87 091	44	34	1	35	19
Sibi	29.55° N, 67.88° E	130	25 700	21	25	12	45	31
Ziarat	30.39° N, 67.71° E	2543	4503	71	34	1	35	19
Kharan	28.58° N, 65.41° E	692	35 630	27	25	10	43	26

masl, meters above sea level; Total HUs, Total housing units; T min, minimum temperature; T max, maximum temperature.

in Northwest Frontier Province, now called Khyber Pakhtunkhwa (Simon Brooker *et al.*, 2004) and the surrounding tribal belt of Federally Administrated Tribal Areas (FATA) (Khan *et al.*, 2016; Qureshi *et al.*, 2016; Irum *et al.*, 2021). CL is also widely distributed in sub-urban localities of Baluchistan province (Bhutto *et al.*, 2009; Ejaz *et al.*, 2008; Hussain *et al.*, 2018), Gilgit Baltistan (Ayub *et al.*, 2003), Azad Jammu and Kashmir (Mughal, 2014; Shaheen *et al.*, 2020). The ZCL type, caused by *Leishmania major*, has its reservoir populations in feral animals predominantly gerbils such as *Rhombomys opimus* in rural and sub-urban localities of Punjab, Baluchistan and Sindh provinces (Bhutto *et al.*, 2003; Afghan *et al.*, 2011).

Leishmaniasis has diverse clinical manifestations and may appear similar to a large variety of other conditions (Afghan *et al.*, 2011; Antinori *et al.*, 2012). CL is identified by the appearance of one or more well-defined ulcerous lesions (Bacellar *et al.*, 2002; Carvalho *et al.*, 2008), usually found on uncovered areas of the body i.e. forearms, legs and face where sand-fly bites occur most often (Salman *et al.*, 1999; Chaudhary *et al.*, 2008). After 4–12 weeks of a bite, a small erythematous papule appears at the bite site which gives off a seropurulent discharge, which later on dries up and then a 'volcanic' nodulo-ulcer appear which is classical for CL (Iddawela *et al.*, 2018). These lesions can develop into other morphological forms such as lupoid, keloidal, psoriasiform, erysipeloid, verrucous, zosteriform, tumorous, eczematoid and acneform (Bari and Raza, 2010; Shamsuddin *et al.*, 2017; Iddawela *et al.*, 2018). Routinely used diagnostic methods are direct clinical observations and micro-scopical examination of lesion aspiration samples. Alternative diagnostic options include histopathology and polymerase chain reaction (PCR) but due to the low frequency of parasites, PCR may give false-negative results. The practice of using PCR as a diagnostic tool is also uncommon worldwide and not easily applied to clinical health settings (Weirather *et al.*, 2011; Tsukayama *et al.*, 2013; de Paiva-Cavalcanti *et al.*, 2015). The sensitivity of enzyme-linked immunosorbent assay is 50% in the case of co-infection due to less circulating antibodies in blood (Sinha *et al.*, 2005; de Souza *et al.*, 2011; Abeijon *et al.*, 2012). The treatment relies on chemotherapy and the first line of treatment for all types of leishmaniasis are the medications containing pentavalent antimonials such as sodium stibogluconate and meglumine antimoniate. But unfortunately, the treatment failure has been reported for this class of drug because the parasite has shown resistance to this class of drug and its authenticity and clinical value has been challenged (Croft *et al.*, 2006; Llanos-Cuentas *et al.*, 2008).

CL is one of the neglected diseases in Pakistan and exact figures on the magnitude of the disease are lacking both at national and regional levels and only a few health centres are available for diagnosis of CL. The limitation/absence in the diagnosis and treatment increase the need for an epidemiological survey of the disease in the country (Afghan *et al.*, 2011). Despite the disease endemicity, the information regarding the epidemiology of CL in Pakistan is incomplete (Khan *et al.*, 2016). Baluchistan is one of the provinces located in the southwest of Pakistan and its geographical distribution has made it a camping ground for sand-fly due to disturbances of the habitats and deforestation (Firdous *et al.*, 2009). Because of the major teaching and tertiary care hospitals located in Quetta, most of CL patients have been reported in Quetta (Kassi and Kasi, 2005). This may be due to mass migration from adjoining areas of Afghanistan and the absence of field epidemiological surveys (Shakila *et al.*, 2006).

The present study was designed with an aim to identify epidemiology and to assess clinical aspects of CL in Baluchistan Province of Pakistan.

Materials and methods

Study area

The study was carried out in different districts of Baluchistan covering the surface area of 347 190 km^2 and constituting 44% of Pakistan's total landmass located between 30.12N and 67.01E. It has borders to Afghanistan from the north and north-west and to Iran from the south-west and has a population of around 10 million inhabitants predominantly (76%) rural. Physically, the topography is diverse and divided into four distinct zones: upper highlands, lower highlands, plains and deserts altitude ranging from 600 m from the valley floor to 3700 m above the mean sea levels. The climate varied from semi-arid summers to semi-arid winter with temperature as high as 50°C and as low as 1°C where the annual average temperature is about 19°C and average annual rainfall for Quetta is 44 millimetres (Kakarsulemankhel, 2004) (Table 1).

Epidemiological investigation

Due to the lack of ground-level research and treatment limitations, the epidemiological status of CL in the study area was not known. Based on university hospital records between August 2018 and December 2019, 4072 cases of CL were reported from the Baluchistan.

Ethics statement

The study was approved by the IRB & Ethics Committee of National University of Medical Sciences (NUMS), Rawalpindi, Pakistan under reference number 06/R&D/NUMS.

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Fig. 1. (a–f) Atypical forms of Cutaneous Leishmaniasis (CL) lesions. (a) Papulonodular lesions, dry type; (b) Ulcerated lesion, dry type; (c) Discoid lupus erythematosus, dry type; (d) Psoriasiform lesion, dry type; (e) Mycetomatous, dry type; (f) Erysipeloid, dry type.

Sample collection and microscopy

A total of 4072 clinically suspected cases of CL visited the Department of Microbiology, Bolan Medical College, Quetta from different areas of Baluchistan. The selected patients were photographed and specific medical explanations for these lesions were obtained from the dermatologist. The samples were collected from the patients after both written and verbal informed consent was obtained. The lesion sample from CL patients was obtained using needle aspiration method. The overlying dry scab was rubbed off with an alcohol gauze pad and approximately 0.1–0.2 mL sterile saline solution injected into the lesion and aspiration fluid was obtained. The slides of smears from each patient were fixed with absolute methyl alcohol and stained with Giemsa stain for parasitological investigation. The slides were examined under the light microscope at 100× magnification for the presence of *Leishmania* spp. amastigotes (Kassi *et al.*, 2004).

Statistical analysis

The CL data were documented into Microsoft Excel and analysed using SPSS (SPSS Inc., Chicago, Illinois, USA) version 26.0. Prevalence of CL in different age groups and gender was calculated. The Chi-square test (χ^2) and frequencies of demographic characteristic were used for categorical data. Monthly distribution of CL cases for the years of 2018 and 2019 was calculated in form of percentage. Moreover, the prevalence of CL by age groups with 95% confidence limits were analysed. The statistical significance was set for all statistical tests at P value <0.05 (two-sided).

Results

In the present study, a total of 4072 individuals comprising both males and females were studied from the period of August 2018 to December 2019 and analysed with respect to age, gender and body parts affected by CL.

Clinical features of CL patients

Of the 4072 CL cases, 50.6% (2062/4072) were positive by direct microscopy. The effect of CL in a different age group is depicted in Fig. 1a–f. The lesions were more prevalent among young individuals as compared to older adults. Exposed parts of the body (hand, cheeks and nose) were the main affected areas. Different clinical forms were observed based on clinical morphology of the CL patients' lesion. These lesions diverge from mild to moderate papulonodular form which is <1 cm in diameter to more severe and complex forms. One of the forms was dry type papulonodular lesions with erythematous smooth and superficial papule on the face and size varied from 0.5 to 1 cm (Fig. 1a). Other form presented the clinically infiltrated ulceration which was ulcerated lesion and present the dry type (Fig. 1b). Among CL patients, lupus erythematosus was most common with unusual presentations ranging from scaly and crusted plaque to enlarged erythematous and infiltrated plaque on the elbow wrapped with white and dry scales mimicking psoriasis (Fig. 1c, d and e). A psoriasiform lesion was also observed on the nose of a CL patient which was characterized by an infiltrated plaque (Fig. 1f).

Age and gender-wise distribution of CL

In the present study, the total number of people infected with CL from August to December 2018 was 969. Of the total, 55.3% (536/969) of them were males while 44.7% (433/969) were females. The highest percentage (38%, 368/969) of CL patients was observed aged between 0 and9 years followed by age group 10–19 years which included 29.6% (287/969) of the cases. However, the lowest proportion (6.9%, 67/969) was observed at 30–39 years old (Table 2).

The total number of people infected with CL during the year 2019 was 3103 with the overall CL infection rate during the year 2019 was higher in males (55.9%, 1735/3103) than females (44.1%, 1368/3103). The highest percentage (40.1%, 1245/3103) was observed at 0–9 years old. Only 28.8% (895/3103) of CL patients were observed in 10–19 years old participants followed

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Table 2. Distribution and prevalence of CL in different age groups and gender for the years 2018 and 2019

				Male			Female		
Year	Age	*n*	(%)	*n*	Positive (%)	*P* value	*n*	Positive (%)	*P* value
2018	0–9	368	38	214	97 (45.3)	0.258	154	72 (46.8)	0.481
	10–19	287	29.6	164	61 (37.2)		123	57 (46.3)	
	20–29	122	12.6	72	25 (34.7)		50	20 (40.0)	
	30–39	67	6.9	24	8 (33.3)		43	17 (39.5)	
	40+	125	12.9	62	21 (33.9)		63	22 (34.9)	
	Total	969		536	212 (39.6)		433	188 (43.4)	
2019	0–9	1245	40.1	695	399 (57.4)	0.035	550	313 (56.9)	0.081
	10–19	895	28.8	510	270 (52.9)		385	205 (53.2)	
	20–29	406	13.1	255	130 (51.0)		151	85 (56.3)	
	30–39	202	6.5	102	48 (47.1)		100	45 (45.0)	
	40+	355	11.4	173	80 (46.2)		182	87 (47.8)	
	Total	3103		1735	927 (53.4)		1368	735 (53.7)	

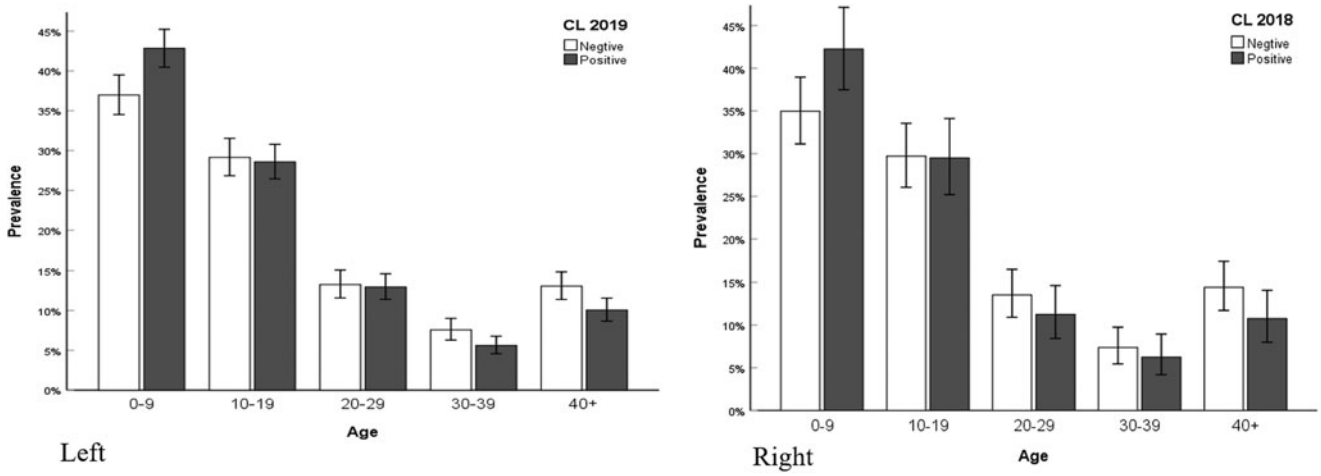

Fig. 2. Prevalence of CL according to gender and age Left: Prevalence of CL by age groups with 95% confidence limits for the year 2019. Right: Prevalence of CL by age groups with 95% confidence limits for the year 2018.

by 13.1% (406/3103) in 20–29 years old, 6.5% (202/3103) in 30–39 and 11.4% (355/3103) in 40+ years old (Table 2). Based on different age groups, the frequency of males to females CL patients was different. Males (22.4%), (16.44%) and (8.22%) were affected more than females (17.72%), (12.41%) and (4.87%) at the age of 0–9, 10–19 and 20–29 years, respectively. On the other hand, the disease was higher in females (3.22%) and (5.87%) than males (0.29%) and (0.58%) at the age of 30–39 and 40+ years as represented in Fig. 2.

Year wise prevalence of CL in different age groups and gender

The findings of the present study revealed that of the total population tested from the period of August–December 2018, 55.3% (536/969) were males and 44.7% (433/969) were females. In total, 41.3% (400/969) of participants were tested positive for lesion aspiration test. Out of which 39.6% (212/536) of males and 43.4% (188/433) of females were positive for lesion aspiration test. The highest percentage (45.3% and 46.8%) in positive lesion aspiration test was found among the participants aged between 0 and 9 years in both males and females, respectively (Table 2). It was observed that the lesion aspiration test was not significantly different by age or gender of participants studied.

In the year 2019, 55.3% (1735/3103) of participants were males and 44.7% (1368/3103) were females. A total of 53% of participants were tested positive for lesion aspiration test and among these 53.4% (927/1735) were males and 53.7% (735/1368) were females. The highest frequency (57%) of lesion aspiration test was tested positive between age 0 and 9 years regardless of age and gender and lowest frequency (47% and 45%) was observed of the participants aged between 30 and 39 years for both males and females, respectively (Table 2).

Site-specific distribution of lesion

The majority 24.4% (757/3103) of lesions were found on the hands followed by face in which cheeks, ears and nose were most affected. The number of lesions per person ranged from 1 to 6 (Fig. 3). Approximately 50% of the participants had single lesion while 14% of the participants had two and nearly 3% of the participants have six lesions during the study period of 2018 and 2019. Most of the lesions were erythematosus with

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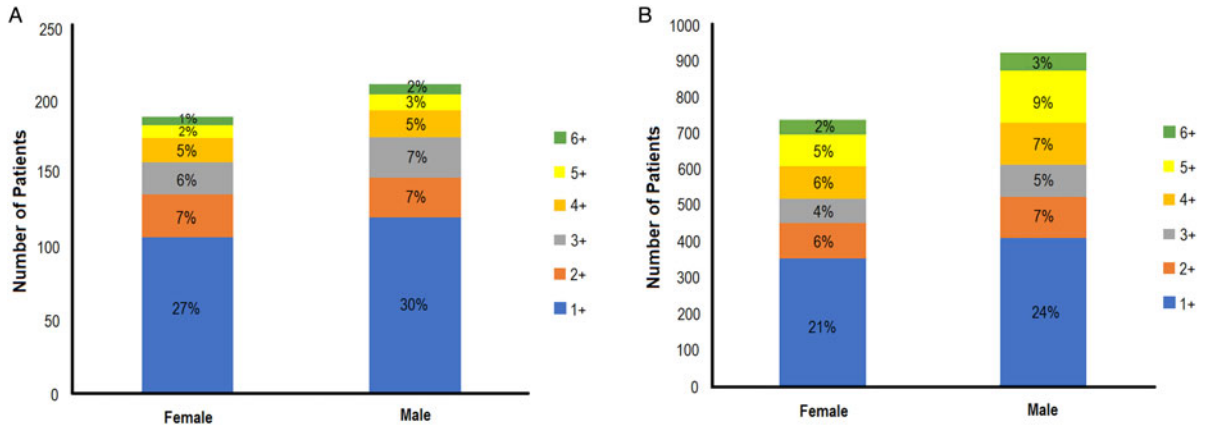

Fig. 3. Number and percentageof male and female patients with active lesions in 2018 and 2019.

unusual presentations ranging from scaly and crusted plaque to enlarged erythematous and infiltrated plaque.

Month-wise distribution of CL

The findings of the study revealed that highest number of CL cases (23.6%, 229/969) was found in December followed by October (22%, 213/969) and August (21.9%, 212/969) during the year 2018. During the year 2019, the highest number of CL cases were observed in May (16.6%, 514/3103) followed by April (13.5%, 418/3103), January (12.5%, 388/3103) and then July (9.6%, 298/3103), February (8.7%, 271/3103) and June (8.5%, 264/3103). The lowest number of CL cases were observed in March (2.7%, 85/3103) and November (3.7%, 116/3103) (Table 3).

Table 3. Monthly distribution of CL cases for the years of 2018 and 2019

Year	Month	*n*	%
2018	August	212	21.9
	September	128	13.2
	October	213	22.0
	November	187	19.3
	December	229	23.6
	Total	969	
2019	January	388	12.5
	February	271	8.7
	March	85	2.7
	April	418	13.5
	May	514	16.6
	June	264	8.5
	July	298	9.6
	August	156	5
	September	163	5.3
	October	216	7
	November	116	3.7
	December	214	6.9
	Total	3103	

Discussion

CL is one of the prevalent vector-borne diseases in Pakistan which affects people with lower income and is linked to poor housing conditions, migration, inadequate nutrition, vector control failures and immuno-compromised conditions like HIV/AIDS (Alvar *et al.*, 2006). CL has been reported from all over Pakistan specially from native surrounding areas of FATA and KPK (Khan *et al.*, 2016; Qureshi *et al.*, 2016; Khan *et al.*, 2019), urban-rural areas of Sindh and Punjab (Iftikhar *et al.*, 2003), Azad Jammu and Kashmir (Mughal, 2014; Shaheen *et al.*, 2020) and sub-urban localities of Baluchistan province (Bhutto *et al.*, 2003; Ejaz *et al.*, 2008; Rahman and Rehman, 2017). It is presumed that uninfected regions with the borders adjacent to endemic areas are at risk and colonization of a large number of populations in areas where sand-fly is endemic is one of the reasons of CL outbreaks (Simon Brooker *et al.*, 2004).

The current study has been conducted in Baluchistan province which is a high-altitude region and borders to Afghanistan and Iran where CL is endemic (Ghatee *et al.*, 2020). The studied area has the highest CL prevalence rate where dominant specie is *L. tropica* and in addition about 1500 cases of CL were reported in Afghan refugee camps in Pakistan in 2002 (Kakarsulemankhel, 2004). In another study Rahman and Rehman (2017) also reported a significantly high level of CL among Afghans compared to the local Pakistani population (32.5% *vs* 20.3%). However, the prevalence of CL due to *L. major* has also been reported from the Southern part of Baluchistan (Bhutto *et al.*, 2009). Thus, the present study was designed for the aim of identifying the epidemiology and assessing the clinical aspects of CL in Baluchistan.

In the current study, CL infection is more prevalent in males (55%) as compared to females with similar findings reported by Shaheen *et al.* (2020) in Azad Jammu and Kashmir, by Galgamuwa *et al.* (2017) in Sri Lanka, by Aara *et al.* (2013) in India by Alavinia *et al.* (2009) and in Iran. It may be due to the reason that activities of the males are mostly out-door and have maximum chances of being bitten by sand fly while females are confined to indoor activities (Gadisa *et al.*, 2015). The current study indicated that 38% of reported CL cases were in children aged 0–9 years followed by adults 10–19 years of age with a prevalence rate of 29% and is in concordance with other studies (Kakarsulemankhel, 2004; Aara *et al.*, 2013; Qureshi *et al.*, 2016; Yohannes *et al.*, 2019; Shaheen *et al.*, 2020). This might be due to a weak immune system and food deprivation of children (Zijlstra, 2016). In contrast to this, Aara *et al.* (2013) reported a higher prevalence of CL cases in 21–30 years age group whereas in our study lower cases of CL were found in 30–39 years old

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and 40+ as also reported by Nawaz *et al.* (2010). As lifelong immunity develops following infection with CL, it is understandable why infection is more common in young individuals.

In the present study, most CL cases appeared to have localized symptoms and most lesions were present on the exposed parts of the body particularly hands, nose, ears and cheeks. Similar results were observed in studies carried out in Ethiopia and Turkey where most of the lesions also appeared on the face (Bari, 2008; Uzun *et al.*, 2018; Yohannes *et al.*, 2019). For the locations of the lesions, it is of the opinion that it is difficult to cover up the face and is exposed for biting of sand flies at night-time which is a crucial time for the parasite spread (Aara *et al.*, 2013). Correspondingly to the findings of Özbilgin *et al.* (2019), 77% lesion appeared on uncovered parts of the body. In the present findings, the common unusual presentations were lupus erythematosus followed by the papulonodular lesion, ulcerated lesion and less common psoriasiform lesion. In CL, atypical clinical presentations were progressively observed in Pakistan and it has been speculated that limitations of these atypical cases in certain geographical regions could be due to new strain of Leishmania parasite (Bari, 2008). In our study, approximately 50% of the CL cases had a single lesion, 14% had two lesions and remaining had three to six lesions. Our results were similar to the findings of Shaheen *et al.* (2020) who also reported the presence of the single lesion in 56% of the CL cases. Other studies conducted by Talari *et al.* (2006) and Qureshi *et al.* (2016) also showed the highest number of single lesions in CL cases. CL cases with two or more lesions on the body might be due to exposure to the sandflies for a long time.

Although CL cases were reported throughout the entire year, the highest number of cases were detected in May, followed by April, January and then July, February and June. The lowest number of cases was during March and November. Similar findings have been reported by Shaheen *et al.* (2020) that warm months are the peak season of CL in Pakistan. This could be due to the reason that sand flies are more active during the warm weather and consumes more blood for the development of their eggs. The current study has shown rising drift in the number of CL cases in Baluchistan and is a disease of public health importance. It is suggestive that different atypical clinical forms of CL depend on host immune response, number and site of parasites inoculated and host nutritional status (Bari, 2008). The study highlights the importance of early detection of erythematic skin lesion and proper management and treatment of CL to prevent a future outbreak of the disease especially in areas where leishmaniasis is endemic. The study also suggests that the use of insecticides, bed nets and new therapies for parasite can also help in the control of leishmaniasis.

Concluding remarks

In conclusion, the current findings suggest that more epidemiological studies are needed in Baluchistan and health education campaigns for population awareness regarding the CL. It is recommended to evaluate the risk factors and to establish control and management strategies to prevent disease at an individual and community level in Baluchistan. It is also recommended to conduct further reservoir studies to understand the vertical transmission of disease among various hosts.

Data. Raw data cannot be made publicly available due to ethical restrictions imposed by the Institutional Ethical Committee on human rights related to research. However, data can be provided on request.

Authors' contributions. HA designed and supervised the study. AK and RS collected the data, drafted and wrote the manuscript along with SN. AH, SG and MTZ contributed to data and sample collection. MQ did the statistical analysis. SS revised the manuscript along with MSA. All authors read and approved the final manuscript.

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Conflict of interest. The authors declare that they have no conflict of interest.

Ethical standards. All procedures performed in studies involving human participants were in accordance with the ethical standards. The study was approved by the IRB & Ethics Committee under project 'A new and combined approach for the diagnosis of cutaneous leishmaniasis, a neglected vector-borne disease' and letter reference number 06/R&D/NUMS.

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