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Coronavirus Pandemic

Incidence of cutaneous Leishmaniasis in humans during the COVID-19 pandemic in Baluchistan Province, Pakistan

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Abstract

Introduction: Cutaneous leishmaniasis (CL) is one of the neglected tropical diseases that affects impoverished communities throughout the world. In Pakistan CL is an endemic disease.

Aims and objectives: This study aimed to determine the incidence of CL infection in the Baluchistan province of Pakistan from January 2020 to March 2022 during the COVID-19 pandemic.

Methodology: A total of 1047 clinically suspected cases of CL from Bolan Medical College Hospital, Quetta, were followed up in the study. The data regarding the epidemiological characterstics, pathological information, and treatment of patients was collected.

Results: Out of 1047 probable cases of CL, 594 (56.73%) cases were found to be positive for CL. Females had the highest infection rate, with the majority of reported cases being in the 0–9-year age group. Most CL cases were reported in April in the year 2020, with a few cases reported in June. But in the year 2021, the highest number of cases were reported in December. The number of overall cases has gradually increased in the year 2022, most likely because of the reduction in COVID-19 pandemic restrictions. The *p* value for the positive as compared to suspected cases in the years 2020, 2021, and 2022 was calculated as 0.8925, 0.8763, and 0.8535 respectively.

Conclusion: Further epidemiological studies and health education campaigns are recommended to increase public awareness. It is strongly advised that local, provincial, and national health authorities establish and maintain effective leishmaniasis surveillance systems to promptly identify disease outbreaks and implement timely control measures.

Key words: Cutaneous leishmaniasis; epidemiology; Baluchistan; Pakistan.

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Introduction

Leishmaniasis caused by the *Leishmania* parasite belonging to the genus Trypanosomatidae, is an infectious disease that spreads through the bite of infected female sandflies to recipient hosts [1]. It ranks among the top three neglected tropical diseases (NTDs) with an estimated 350 million people at risk, and 1.3 million new cases reported every year [2,3]. Leishmaniasis is endemic in many regions including the Mediterranean basin, Africa, Asia, and Latin America [4]. At least 20 species of *Leishmania* are reported to cause a wide variety of clinical manifestations, ranging from asymptomatic infection to severe and potentially fatal disease. The most common variants of the disease are cutaneous leishmaniasis (CL), oral or mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) [5,6]. CL is generally distributed in North America and South America, the Mediterranean region, and Western Asia with between 0.7-1.2 million cases per year [7]. The nations most affected by CL are

Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, Peru, North Sudan, and Syria, comprising approximately 75% of the worldwide incidence [8-9].

CL remains one of the most significant and rapidly growing public health concerns in Pakistan, especially in areas bordering Afghanistan and in cities where an influx of Afghan refugees has been documented [10]. Zoonotic (ZCL) and anthroponotic (ACL) forms of CL caused by *L. major* and *L. tropica*, respectively, have been reported in these areas [11]. The pathogen is most widely distributed in rural and urban areas of Pakistan including Baluchistan, Punjab, Sindh, Azad Jammu Kashmir (AJK), Khyber Pakhtunkhwa (KP), and its neighboring tribal belt, known as the Federally Administrated Tribal Areas (FATA) [12-15]. Given Pakistan's limited diagnostic capacity, it is likely that the reported 50,000 national cases of CL during 2019 are an underestimate. There are few treatment facilities in Pakistan, and they are dispersed unevenly across the country, making access difficult for those in more remote communities. Prior to the COVID-19 pandemic in early 2020, clinics in Pakistan reported > 250 new patients per week for CL treatment [16].

The hallmark of CL is the classic sore in which metastatic cutaneous lesions develop [17]. However, the clinical manifestations of CL can be highly variable with unusual signs being fissures on lips, lupoid lesions on the face, and psoriasiform plaques on the nose [18,19]. Atypical presentations, either due to lesion sites or unusual morphology of the disease, have also been reported in many studies from Pakistan. The most commonly employed diagnostic approaches are visual examination by an experienced clinician and microscopic examination of lesion aspirations [20]. Histopathology and polymerase chain reaction (PCR) are alternative diagnostic methods, although, in patients with low parasite load, other methods of diagnosis may serve as less sensitive as compared to the PCR [21,22]. A Real-Time PCR designed assay can give much better results for the diagnosis of leishmaniasis as compared to the conventional PCR approach [22]. Chemotherapy is used to treat all forms of leishmaniasis. The drugs containing pentavalent antimonial compounds, remain the first line of treatment. Unfortunately, therapeutic failure for this class of therapy has been reported because the parasite has acquired resistance to this class of drugs [23,24].

Leishmaniasis remains an endemic public health problem in Pakistan due to insufficient testing and reporting. A formal program of surveillance should be considered locally and nationally. Even though leishmaniasis and COVID-19 have distinct pathophysiology, the overlapping clinical characteristics pose a challenge, particularly in terms of timely identification and treatment. Immunocompromised people are particularly sensitive to both infections, and co-infection is associated with an increased risk of death. However, during the time of COVID-19, a rather gradual decrease was observed with regard to overall cases. CL is endemic in Baluchistan as its geographical location is ideal as a breeding ground for sandflies due to habitat disruptions and deforestation. It is positioned at the border of Afghanistan and Iran and has one of the highest incidences of CL in the country. The majority of CL cases documented during the COVID-19 epidemic from January 2020 to February 2022 were in the tertiary care hospital of Quetta. People belonging to almost all age groups are susceptible to CL, however children are specifically prone to CL. One of the likely reasons is their outdoor habits of playing and contacting the CL vector, the sand fly. Another important reason of higher CL rate among children is because of their weekend immunity. People with COVID-19 already have compromised immunity, so they are more susceptible to CL infection.

The current study was designed to elucidate the epidemiology of CL in Baluchistan during the COVID-19 pandemic from January 2020 to March 2022, when resource distribution and access to medication were severely impacted. The study also aimed to understand how circulating virus may affect the transmission of *Leishmania*.

Methodology

Ethics statement

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

Study area

The study was carried out in Baluchistan which is Pakistan's largest province in terms of land area but also the least populated. The provincial capital and largest city is Quetta. Baluchistan shares borders with Punjab and the Khyber Pakhtunkhwa to the northeast, Sindh to the east and southeast, the Arabian Sea to the south, Iran to the west, and Afghanistan to the north and northwest. Aside from Quetta, the second-largest city of the province is Turbat in the south, while another area of major economic importance is Gwadar Port on the Arabian Sea. The climate ranges from semi-arid

summers to semi-arid winters, with temperatures as high as 50°C in the summer season and as low as −10°C, in winter season [25]. The study area map is shown in Figure 1.

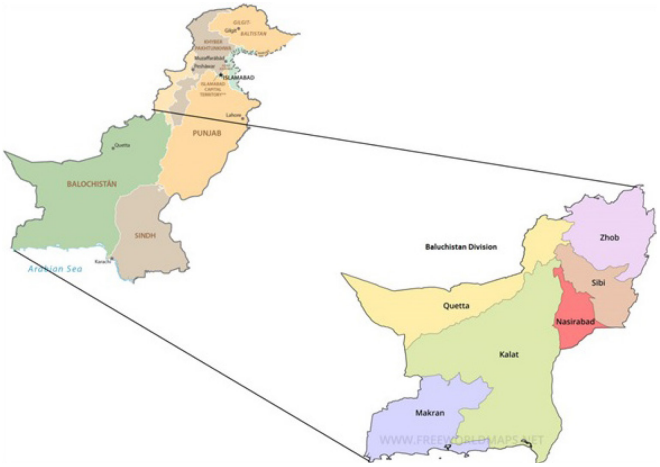
Epidemiological investigation

The epidemiological data concerning CL patients during the COVID-19 pandemic covering the period of January 2020 - March 2022 was collected from Bolan Medical College Hospital, Quetta. According to the hospital records, 1047 probable cases of CL were reported for treatment from different areas of Baluchistan during this period.

Sample collection and microscopy

In all the 1,047 probable cases of CL visiting the Bolan Medical Complex in Quetta, the needle aspiration method was used to obtain a lesion sample. Before performing needle aspiration, the dry surface of the lesions was scraped away with a spirit swab, and then 0.2 mL of normal saline was injected into the lesion. For microscopy, slides with the lesion aspirate were fixed with 100% methyl alcohol and stained with Giemsa stain. The slides were then analyzed for the presence of *Leishmania* spp. amastigotes under a light microscope at 100 × magnification [26].

Figure 1. Study area map of Baluchistan and adjoining regions of Pakistan.



Statistical analysis

After importing the patient data into MS Excel, data were analyzed using version 26.0 of SPSS (SPSS Inc., Chicago, Illinois, USA) to determine the presence of *Leishmania* parasites by age group and gender. For categorical data, the Chi-square test (χ^2) and demographic feature frequencies were performed. The percentage monthly distribution of CL cases from January 2020 to March 2022 was calculated. Furthermore, the age-specific prevalence of CL was

Table 1. Distribution of probable and confirmed cases of CL.

Year	Demographics	Parameters	Lesion			Degree of Freedom	Chi square	*p*-value
			N	Infected	(%)			
2020	**Gender**	Male	408	193	47.30	1	2.0118	0.1561
		Female	323	183	56.65			
	Age (Years)	0-10	327	182	55.65	6	2.277	0.8925
		11-20	182	91	50.00			
		21-30	115	50	47			
		31-40	48	25	52.08			
		41-50	34	16	47.05			
		51-60	18	7	38.89			
		> 60	7	4	57.14			
		Total	**731**	**376**	**51.43**			
2021	**Gender**	Male	101	63	62.37	1	1.0778	0.0588
		Female	82	65	78.31			
	Age (Years)	0-10	60	45	75.00	6	2.43	0.8763
		11-20	52	34	65.38			
		21-30	31	21	67.74			
		31-40	20	14	70.00			
		41-50	9	7	77.77			
		51-60	6	3	50.00			
		> 60	5	4	80.00			
		Total	**183**	**128**	**69.94**			
2022	**Gender**	Male	72	47	65.27	1	0.5987	0.4391
		Female	61	49	80.32			
	Age (Years)	0-10	55	49	89.09	6	2.6312	0.8535
		11-20	37	21	56.75			
		21-30	11	7	63.63			
		31-40	9	6	66.67			
		41-50	11	8	72.72			
		51-60	7	4	57.14			
		> 60	3	1	33.33			
		Total	**133**	**96**	**72.18**			

examined with 95% confidence intervals. The statistical significance level was set at 0.05 for all statistical tests.

Results

Annual prevalence of probable and confirmed cases of CL

In the present study, 57% (594/1047) of the total population were confirmed as positive for CL over the 3-year period. In the year 2020, 51.43% (376/731) of the cases tested positive; of these, 47.30% (193/408) were males and 56.65% (183/323) were females. The highest number of cases were reported from the age group 0-10 years old (182/327). The prevalence of diseases in females was found to be greater i.e., 183/323 (56.65%) as compared to males 193/408 (47.30) (Table 1).

Positivity for CL during 2021 increased to 69.94% (128/183) with the overall infection rate in females being 79.27% (65/82) and 62.38% (63/101)in males. The highest number of CL-positive patients 26/31 (83.87%) were observed in the 0–10-year aged females group and 21-30 aged females group (12/14, 85.71%) respectively. In the age groups 41-50 (females) and 51-60 (males), only a few reported patients (being 5 females and 2 males) were all found to be positive (Table 2).

From January to March 2022, CL positivity further increased to 72.18% (96/133) with the overall infection rate in females being 80.32% (49/61) and 65.27% (47/72) in males. The highest number 26/30 (86.6%) was observed in the 0-10 years age group for males and females 23/25 (92%) as well. This is followed by 14/17 (82.35%) in the 11-20 year group (females) (Table 1).

Age and gender-specific distribution of CL

From January to December 2020, the total number of probable cases with CL was 731, of which 55.81% (408/731) were males and 44.18% (323/731) were females. A total of 51.43% (376/731) of these tested positive for CL on lesion aspirate microscopy. Of these CL-positive individuals, 47.30% (193/408) were males and 56.65% (183/323) were females. The lowest number of cases were reported in the age group 51-60 years (males) with a percentage of 27.27% (3/11). The lowest number of cases were reported in females greater than 60 years of age with the percentage 33.33% (1/3). The trend for cases was seen to be decreased with age, overall (Table 2).

During the year 2021, the total number of individuals suspected to have CL infection were 183, out of which 128 were found to be positive (69.94%). Among the affected ones, 62.37% (63/101) were males, and 78.31% (65/82) were females. The females with age groups 0-10 years and 21-30 years were affected more as compared to the other age groups, and males as well.

In 2022, the total number of probable cases with CL infection increased more. More number of CL patients were observed in the year 2022, as compared to the years 2020 and 2021. The reported figures have shown

Table 2. Prevalence of CL by age and gender among probable cohorts.

Year	Age	Male						Female					
		n	Positive	(%)	df	Chi square	*p*-value	n	Positive	(%)	df	Chi square	*p*-value
2020	0-10	159	85	53.46	6	3.4499	0.7506	168	97	57.74	6	2.0699	0.9132
	11-20	105	51	48.57				77	40	51.95			
	21-30	73	30	41.09				42	20	47.62			
	31-40	31	13	41.94				17	13	76.47			
	41-50	25	8	32.00				9	7	77.77			
	51-60	11	3	27.27				7	5	76.47			
	> 60	4	3	75.00				3	1	33.33			
	Total	**408**	**193**	**47.30**				**323**	**183**	**56.65**			
2021	0-10	29	19	65.52	6	3.1446	0.9973	31	26	83.87	6	9.7913	0.7109040.710904
	11-20	38	23	60.53				14	11	78.57			
	21-30	17	9	52.94				14	12	85.71			
	31-40	10	7	70.00				10	7	70.00			
	41-50	4	2	50.00				5	5	100.00			
	51-60	2	2	100.00				4	1	25.00			
	> 60	1	1	100.00				4	3	75.00			
	Total	**101**	**63**	**62.38**				**82**	**65**	**79.27**			
2022	0-10	30	26	86.67	6	3.8983	0.6904	25	23	92.00	6	0.8171	0.9916
	11-20	20	7	35.00				17	14	82.35			
	21-30	5	3	60.00				6	4	66.67			
	31-40	6	4	66.67				3	2	66.67			
	41-50	7	5	71.42				4	3	75.00			
	51-60	3	2	66.67				4	2	50.00			
	> 60	1	0	00.00				2	1	50.00			
	Total	**72**	**47**	**65.27**				**61**	**49**	**80.32**			

that 65.27% (47/72) males were affected, and 80.32% (49/61) females were found to be positive. The highest number of CL positive cases was found in the 0-10 year age group for both males and females with percentages of 86.67% (26/30) and 92% (23/25), respectively (Table 2). Based on different age groups, the incidence of male-to-female CL-positive cases was different (Table 2).

Monthly distribution of CL

In the year 2020, the maximum number of CL-positive cases (66.7%) was observed in April, followed by May (64.3%), August (60%) and January (58.06%) respectively. During 2021, the maximum number of positive cases was diagnosed in December (86.79%) followed by November (76.01%) and October (60.42%) respectively. The highest number of cases during 2022 was observed in January (76.92%) followed by February (70.27%) and March (57.1%) (Table 3 and Figure 2). Overall, the highest number of cases have been reported in the month of December, in the year 2021, as compared to 2022 or 2020. But the incidence of cases gradually increased in the year 2022, as concomitant with the COVID-19 pandemic's downfall.

Discussion

CL exists as a complex epidemiological triad, with various epidemiological risk factors associated with the host, vector, and environment. Early detection of such risk factors may help to prevent further transmission to vulnerable populations [27]. CL is widely distributed throughout Pakistan with its continuous spread in border areas, especially in Baluchistan. Parasitic colonization of a large number of sandfly populations in endemic areas is one of the causes of CL outbreaks [28]. The high prevalence rate of CL is due to *L. tropica* being the dominant species. However, CL caused by *L. major* has also been reported in southern Baluchistan [12,29] with an estimated 4072 active cases of CL from various parts of Baluchistan during 2018-2019. Another study also reported the dominance of CL in three rural areas of Baluchistan i.e., Winder, Uthal, and Lasbella with a large number of sandflies observed during the hot weather [30].

In the current study, the distribution of CL was observed across every age group, but mainly the highest number of CL cases throughout was recorded in children aged between 0-10 years (Figure 3). This phenomenon has been evident in other studies, where

Figure 2. Monthly prevalence of CL from 2020 to 2022.

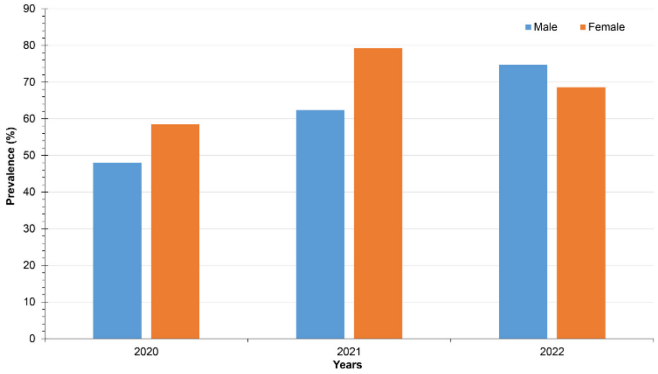


Figure 3. Age-specific prevalence of CL.

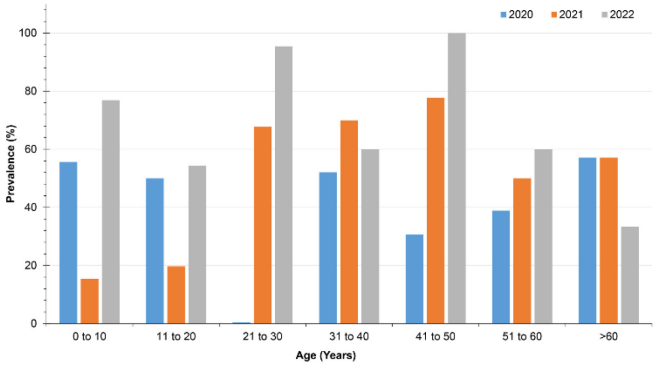


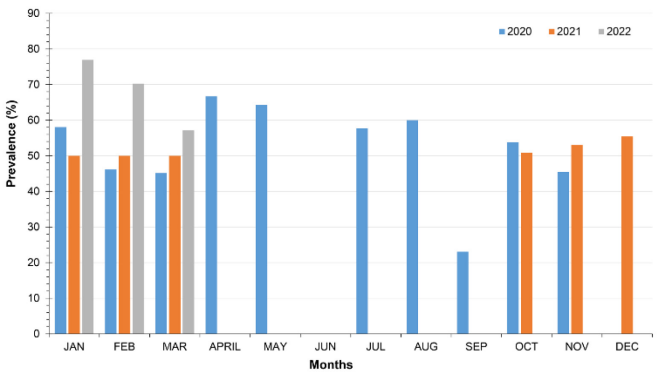
Table 3. Month wise prevalence rate of Leishmaniasis.

Year	Months	January	February	March	April	May	June	July	August	September	October	November	December	Total
2020	Infected	162	137	28	4	9	0	15	6	3	7	5	0	376
	Total	279	297	62	6	14	0	26	10	13	13	11	0	731
	(%)	58.06	46.13	45.16	66.67	64.29	0	57.69	60.00	23.08	53.85	45.45	0.00	
Statistical analysis							df: 11; χ^2: 15.79							
2021	Infected	8	8	2	0	0	0	0	0	0	29	35	46	128
	Total	16	16	4	0	0	0	0	0	0	48	46	53	183
	(%)	50.00	50.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00	60.42	76.01	86.79	
Statistical analysis							df: 11; χ^2: 19.67							
2022	Infected	40	52	4										96
	Total	52	74	7										133
	(%)	76.92	70.27	57.14										
Statistical analysis							df: 11; χ^2: 0.25							

children are more likely to be bitten by sandflies and develop CL infection because of their outdoor habits. Children are one of the most vulnerable groups for infection and disease progression could be due to weakened immunity and malnutrition in these children [29,31-33]. The infection is found to be more abundant in females in comparison to males (Figure 4) with similar findings reported by a study conducted in Yemen [34]. Contrary to that, another study carried out in Baluchistan has shown that 55% of the males were infected more, compared to the females [29]. The studies conducted in Azad Jammu and Kashmir, Sri Lanka, India, and Iran reported a high prevalence of CL in males compared with females [28,35,31,36]. One of the most important reasons for the spread of CL disease in Baluchistan is the migration of Afghan refugees (different age groups) from Afghanistan to Baluchistan Pakistan, as the neighboring Afghan border is close to the Baluchistan. So these refugees carrying the CL disease are also the source of transmission to the people living in Baluchistan [30]. The present study also demonstrates that the frequency of CL cases was increased in the year 2021 (Figure 4) and then 2022, in relation to 2020. It may be ascribed to the reduction of COVID-19 pandemic in the year 2022 specifically, as in the years 2020 and then 2021, there have been lockdowns and closure. So COVID-19 pandemic restrictions caused the low number of cases to be reported from the study area. In the year 2022, COVID-19 pandemic's downfall was seen and that's the likely reason behind the incidence of many cases during the period of COVID-19 emergency in the country, emergency care to other diseases could not be administered as well.

The seasonal pattern of disease transmission can be used to plan disease surveillance and control activities. Patients with leishmaniasis who were enrolled in the current study visited the hospital for a total of 12 months in 2020, 2021, and 2022. During the year 2020 in spring (January – May) and in the months (July – November), there was a considerable increase in patient visits to the hospital. In 2021, from January to March and October to December, there was a considerable increase in the number of infections. There were no CL cases reported from April to September 2021, which was peak season of COVID-19 infection in Baluchistan and outpatient department (OPDs) were classified specifically for COVID-19 patients. Many cases were reported from January to March in 2022. Similar findings have also reported that autumn and summer are the peak seasons for CL in Pakistan [29,14,27]. Our study demonstrates that the sand-fly vector's seasonal activity from January to March is constant over these three years. The monsoon season lasts from June to September and results in heavy and prolonged rainfall, whereas the dry season lasts from November to January. Different studies have identified various transmission patterns, indicating seasonal tendencies in various geographical regions [37,25]. High temperature in Baluchistan is the likely factor supporting high growth of parasite harbouring sand flies [30]. During the time period of COVID-19 spike in Pakistan, many other vector borne diseases along with CL, i.e, Malaria, Dengue Virus, Chikungunya virus emerged and strained on already failed healthcare system. The high similarity between these diseases put another confusion between their diagnosis during the challenge of COVID-19 [38].

Figure 4. Prevalence of CL by gender and year.



This study found an increasing tendency in the number of CL cases in Baluchistan, indicating the emerging public health importance of this disease. The increase in clinic visits with a CL diagnosis in Baluchistan during the pandemic period from 2020 to 2022 demonstrates that the country's epidemiological surveillance of neglected tropical diseases must be consolidated, and even upgraded. The study emphasises the importance of the management and treatment of CL to prevent future outbreaks, particularly in endemic regions. The study also points towards the use of insecticides, bed nets for children in particular, and new parasite therapies to augment the control of this disease.

Conclusions

This study implies that further public health-related investigations and health education efforts in Baluchistan are needed to raise public knowledge about leishmaniasis. Furthermore, the study advocates that due to the COVID-19 pandemic, there is a relative decrease in the reporting of CL cases. As according to previous study of 2018-2019, the active cases were found to be 4072, however, during the COVID-19

pandemic time, these were found to be 594. During the pandemic, COVID-19 prompted an emergency in country preventing these cases from being highlighted. So, when the situation became better, the cases started to increase, as the present study also validates, in the year 2021 and then 2022.

In Baluchistan, it is advised that risk factors be assessed, and control and management techniques be used to avoid illness at all levels. Further reservoir studies are also required to better understand disease vertical transmission among varied hosts. Health authorities should form a leishmaniasis control group in collaboration with the Ministry of Health. It is strongly suggested that health authorities at the local, provincial, and national levels establish and maintain effective leishmaniasis surveillance systems to promptly identify leishmaniasis outbreaks and undertake control measures in a timely manner.

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Authors' contributions
S.A.B. and L.S. collected the data and authored the paper following discussions with S.G., A.H., N.A.S., H.A., and J.C. H.A. designed the study. H.A., S.N., E.D., Z.A., L.D., Y.W., and J.C., revised the paper. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The data presented in this study has been provided on request of the corresponding author.

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