"Epidemiology and distribution of cutaneous leishmaniasis in District Dera Ismail Khan, Pakistan"

BY

AQSA MANSOOR

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ISLAMABAD PAKISTAN

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"Epidemiology and distribution of cutaneous leishmaniasis in District Dera Ismail Khan, Pakistan"

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN ZOOLOGY

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بسم الله الرحمن الرحيم

DEDICATION

"I am honored to dedicate my research to my Parents."

ACKNOWLEDGEMENT

All praise belongs to Allah, the creator of the universe. The Most Compassionate, the Most Generous and Merciful, who is the sole source of wisdom and knowledge given to mankind, who provided me the strength and capacity to pursue this objective, and Who, in my opinion, never wastes an effort made in doing good actions. Glory of Allah be upon His Prophet Muhammad (PBUH) & Holy Family of Prophet Muhammad (SWA), who has led his Ummah in knowledge-seeking from birth to death.

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AQSA MANSOOR

DECLARATION

It is hereby declared that the material contained in this thesis " Epidemiology and distribution of cutaneous leishmaniasis in District Dera Ismail Khan, Pakistan " is my original work. No part of this thesis has been previously presented for any other degree.

AQSA MANSOOR

February 2023

CERTIFICATE

This is to certify that the dissertation entitled **"Epidemiology and distribution of cutaneous leishmaniasis in District Dera Ismail Khan, Pakistan"** submitted by **Aqsa Mansoor** is accepted in its present form by the Department of Zoology, Quaid-I-Azam University Islamabad Pakistan, as satisfying the dissertation requirement for the degree of MPhil in Zoology.

Supervisor: ________________________________

Dr. Kiran Afshan

External Examiner: _________________________

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Dr. Amina Zuberi

Chairperson

Dated: 01-02-2023

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List of abbreviations

Abbreviations	Full form
CL	Cutaneous Leishmaniasis
VL	Visceral leishmaniasis
MCL	Mucocutaneous leishmaniasis
DCL	Diffuse leishmaniasis
ACL	Anthroponotic leishmaniasis
ZCL	Zoonotic leishmaniasis
OW	Old World leishmaniasis
NW	New World leishmaniasis
DNA	Deoxy Ribo Nucleic Acid
PCR	Polymerase chain reaction
WHO	World Health Organization
MSF	Medecins Sans Frontiers
FATA	Federally Administered Tribal Areas
KPK	Khyber Pakhtunkhwa
D.I.K	Dera Ismail Khan
SPSS	Statistical Package for Social Sciences
GIS	Geographic Information System
ArcGIS	Aeronautical Reconnaissance Coverage-Geographic Information System
DEM	Digital Elevation Model
IDW	Inverse density weight
API	Annual Parasitic Incidence
LST	Leishmania skin test
IM	Intramuscular infusion
IV	Intravenous infusion
LHM	Local Health Ministries
DHQ	District Headquarter
THQ	Tehsil Headquarter

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ABSTRACT

Background: Leishmaniasis is a parasitic infection that affects people in tropical and temperate regions. It is spread by the biting of sand flies, particularly *Phelotomus* and *Lutzomyia*. Sindh, Punjab, Baluchistan, and Khyber Pakhtunkhwa (KP) have higher rates of Cutaneous Leishmania (CL). Environmental variables influencing CL endemic locations in Pakistan are not understood clearly. The current study was aimed to determine the prevalence of cutaneous leishmania and to develop risk map for predicting CL distribution in Khyber Pakhtunkhwa, Pakistan.

Material and Methods: A total of 1135 clinically verified subsequent cases of cutaneous leishmaniasis from January 2019 to March 2022 were included in this investigation. Using fine needle aspiration, the diagnosis was validated. Environmental and clinical data from DHQ D.I.K and other local CL centers were collected. By mapping the data using ArcGIS version 10.8 and Google Earth Pro version 7.3.0, the spatiotemporal prevalence of CL infection was examined.

Results: Cutaneous leishmaniasis was recorded highest 65% among individuals aged under 30 years. The male cases were high 61.7% than females 38.3%. Early and late lesions were classified from less than two months to more than a year, highest lesion duration between 1-2 months was 57.1%. A total of 1204 lesions, out of these 76.1% individuals fall in category of single lesion while 23.9% fall in multiple lesions. Most of the lesions seen on exposed body areas such as lower extremity was 34.2%, face 30.7%, upper extremity 29.1% and a least number of mixed lesions was 6.1%. Lesions were of nodular and ulcer type. In spatiotemporal analysis, a total of 989 CL cases were recorded in all villages. A choropleth map showed that Tehsil-wise increased incidence of CL at D.I.K with reported cases 63% followed by Paharpur 10%, Paroa 7%, Kulachi 6% and Daraban 2%. An elevation map of the average incidence of CL plotted on DEM, showed that high altitudes have a lower prevalence of CL. The future epidemic threats of CL infection were predicted by IDW map and D.I.K, Kulachi and Paharpur were CL risk areas. The high cluster counties according to the cluster and outliers study were D.I.K and Paharpur ranging from 42750.0 m (z-score= 1.8, P=0.071397) to 51300.0 m (z-score= 1.4, P=0.142513) and Kulachi, Daraban and Paroa ranging from 17100.0 m (z-score= 1.0, P=0.273971), 25650.0 m (z-score= 1.4, P=0.150079) and 34200.0 m (z-score= 0.9, P=0.318580) respectively, were the high and low outlier villages.

Conclusion: Leishmaniasis is prevalence was high among the local population, but a temporal increasing pattern was seen in the tehsil D.I.K. and Paharpur, which suggests a potential danger for the spread of CL. For disease prevention and management at the individual and community levels, the area needs to get the right attention.

INTRODUCTION

Leishmaniasis is a zoonotic, anthroponotic and vector-borne disease, which is caused by protozoan parasites and transmitted by infected female phlebotomine sand flies *diptera phycodidae* (Cassagne et al., 2014). Leishmaniasis has mainly three clinical presentations that are: visceral Leishmaniasis (VL)-commonly known as kala-azar, mucocutaneous Leishmaniasis (MCL) and cutaneous Leishmaniasis (CL). Unlike visceral and mucocutaneous Leishmaniasis, cutaneous Leishmaniasis is not as deadly as VL and MCL, and is the common form of disease, while visceral Leishmaniasis (VL) is almost fatal if it is left untreated (Akuffo et al., 2021). The clinical symptoms of the disease's progression depend upon the immunological response of the host and the affecting specie. Cutaneous Leishmaniasis, depending upon the either of the form, non-ulcerative nodules, ulcerative nodules or destruction of baso-buccal mucosal tissue could occur (Laynez-Roldán et al., 2020; Valero & Uriarte, 2020). Leishmaniasis is a serious health issue in many native countries. Whereas in past decade, a continuous increase has been reported(Aissaoui et al., 2021). There are two main types of Leishmaniasis old world and new world *Leishmania*ses. The old world *Leishmania* specie spreads through the blood stream in the body and it affects the internal organs, which in turn manifests as VL, whereas the CL is confined to skin only. However, the new world Leishmaniasis specie causes CL and MCL with the involvement of skin as well as mucous membrane (Azim et al., 2021).The infected phlebotomine sandflies that transmit *Leishmania* parasites and 98 other species of genera *Phlebotomus* and *Lutzomyia* sand flies have been considered as proven or suspected vectors for the transmission of Leishmaniasis in the human population (Maroli et al., 2013). The phlebotomine sand flies carry old world Leishmaniasis specie(Figure 1.1), whereas the *Lutzomyia* vector carries new world Leishmaniasis species (Lafri & Bitam, 2021).

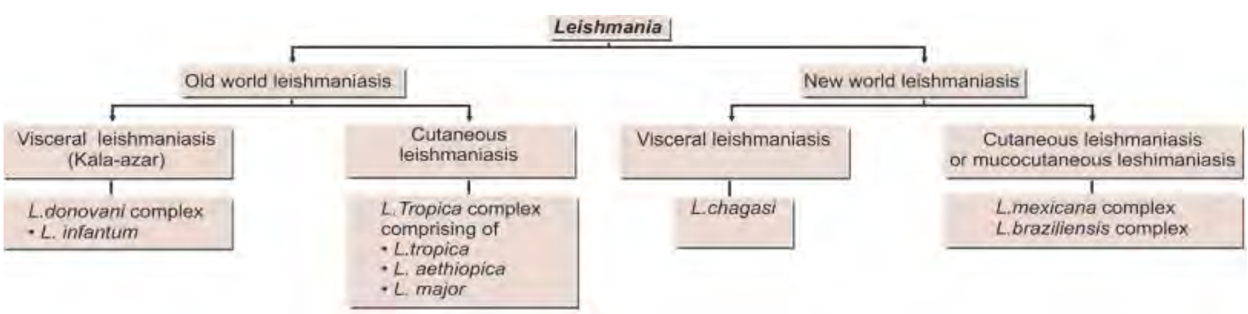

Figure 1.1: Old world and new world Leishmaniasis (Paniker & Ghosh, 2017).

1.1 Global Impact of Leishmaniasis

Female sandflies attack mammals to take their blood meals. For the completion of egg development some sandflies feed on humans while others feed on wide variety of hosts including Canidae, marsupial, rodents and hyraxes (Steverding, 2017). The clinical effects of the disease are determined by various factors such as virulence, specie of *Leishmania* parasite and host immune responses, resulting in CL, MCL, and VL. *Leishmania tropica* and *Leishmania major* are the prevalent species causing old world Leishmaniasis. *Leishmania* aethiopica can cause the acute mucocutaneous and diffuse variants, which are limited to Eastern Africa. The new world CL is causal by the *Leishmania braziliensis* and *Leishmania Mexicana* complexes. *Leishmania donovani* is the most important species responsible for VL (Table 1.1) (Aissaoui et al., 2021; Ameen, 2010). After malaria, Leishmaniasis is the most deadly and neglected protozoan disease, reported by World Health Organization (WHO). They have strong and complex relationship with poverty, as it affects mainly poverty-stricken rural and sub-urban areas, having large population, poor housing, lack of resources and weak immune system of masses. However, in recent years it has been spread to urban populations as well (Moradi et al., 2021). According to world health organization (WHO), this disease exists in 98 countries with an estimated date toll of 20,000-40,000 and 12 million cases at risk per year as reported in 2020. The yearly prevalence of new cases for CL and VL ranges from 0.7-1.2 million and 0.2-0.4 million cases respectively (Sabzevari et al., 2021). In 2018, approximately 85% of novel cases of CL happened in 10 countries that are; Algeria, Bolivia, Brazil, Colombia, Iraq, Pakistan, Tunisia, Syria and Iran, with a highest emergence of CL cases in Iran (Motalleb et al., 2021).

Numerous risk factors that increase the prevalence and incidence of Leishmaniasis are mostly man-made. In general, the main threats to anthroponotic life are socioeconomic status, environmental conditions, demographics, and human behavior (Oryan & Akbari, 2016). More than 67% of the worldwide incidence of VL is mostly reported among Indian residents. In India, Nepal, and Bangladesh, the number of people at risk ranges from 200 to 300 million and the annual financial impact is probably up to US $350 million (Pavli & Maltezou, 2010).

The third clinical form, MCL, is caused by *Leishmania Vianna braziliensis* (LVB), mostly in the south and central American region. Mucocutaneous Leishmaniasis, the new world disease is mostly reported from Brazil, Peru, and Bolivia. The involvement of the oral mucosa is considered sporadic and only occurs in 3-5% of infected patients with LVB (Botelho *et al.*, 2021). Even if it's being treated, muco-cutaneous and diffuse cutaneous lesions can lead to deadly secondary infections. People with weak or compromised immune system are more vulnerable to CL, however, in Latin America, it is being associated with region strains of *L.Mexicana* and *L.braziliensis* species complexes (Ready, 2010).

Table 1.1: The major *Leishmania* species for human Leishmaniasis.

Clinical presentation	**Location**	**Species**
CL	OW	*L.Tropica, L.major ,L.aetiopica*
		(Rarely MCL/DCL)
		L. Mexicana complex
	NW	*L.Venezuelensis*
		L. Amazonesis
		(Rarely DCL)
		L.Mexicana
CL/MCL	NW	***L. braziliensis complex***
		L. peruviana
		L. braziliensis
		L. guyanensis
		L. colombiensis
		L. panamensis
VL/CL	OW	***L. donovani complex***
		L. donovani
		L. infantum
	NW	***L. donovani complex***
		L. chagasi

NW: New World, OW: Old World, DCL: Diffuse CL.

1.2 Cutaneous Leishmaniasis in Pakistan

In Pakistan, both cases of cutaneous and visceral Leishmaniasis are being found. In the northwestern region of the country, a 2.7% prevalence rate has been reported (Azim et al., 2021).

While in Iran anthroponotic cutaneous Leishmaniasis (ACL) and Zoonotic cutaneous Leishmaniasis (ZCL) are the two main types of CL, which are caused by L. major and L. tropica, respectively. Some species of sand flies, such as *Phlebotomus papatasi* and *Phlebotomus sergenti* are the main vectors for ACL and ZCL in Iran, respectively (Soltani et al., 2019).Leishmaniasis affects mostly people of low socioeconomic status, linked with mud-made housing, weak immune systems, and a lack of financial resources. Similarly, Climate change, rural-urban labor migration, and civil unrest continue to be significant risk factors for ACL (Bamorovat et al., 2021). Leishmaniasis has been reported in the animal and human populations in Pakistan. Human-CL is prevalent in some parts of Pakistan and, after malaria, it is the second most endemic vector-borne disease in the country. In Pakistan, 37 species of sand fly are being responsible for the transmission of the disease to healthy hosts (Kayani et al., 2021). Phlebotomine sand flies are the main carrier for the transmission of CL. *L. tropica* while *L. major* are the main causative parasitic species in the region (Jacobson, 2003). *L. major* is zoonotically transmitted by mammals such as gerbils and jirds, which are considered the main reservoir hosts in rural, suburban and lowland regions, while *L. tropica* is transmitted anthropologically in the urban and highland areas (Kaemingk et al., 2019). CL is the most prevalent and common type of Leishmaniasis, which is characterized by ulcerative skin lesions that usually self-heal over a period of time ranging from a month to a year (Zabala-Peñafiel et al., 2020).

In Pakistan, cutaneous Leishmaniasis is mostly prevalent in the northern regions, southern Punjab, Sindh and Baluchistan. In the province Baluchistan, specifically Quetta and surroundings are severely affected by CL due to limited access to health care centers being an underprivileged and poor region (Ullah et al., 2020).In Pakistan, round about 21,000-35,000 cases of both zoonotic and anthroponotic types of CL are being reported. The ZCL form is more common and attributed to infection by L. major, which is mostly reported from semi-urban and rural areas of Sindh, Baluchistan, and Punjab provinces, where its transmission is continued, probably through reservoir populations of wild mammals, mainly gerbils such as *Rhombomys opimus* (Ullah et al., 2020). The human is the only source of infection for the carrier in ACL (Zaidi et al., 2017). Anthroponotic cutaneous Leishmaniasis is clearly sporadic, and L. tropic has been identified as the disease's causative agent in the country. It is mostly recorded in Baluchistan, Punjab, Azad Jammu and Kashmir (AJK), Khyber Pakhtunkhwa and the adjacent tribal zone referred to as Federally Administered Tribal Areas (FATA) (Ullah et al., 2020). *L. tropica* causes ACL, which is reported from all over Pakistan, with most cases in Khyber Pakhtunkhwa province. In many districts of the Khyber Pakhtunkhwa(KPK) province, the ACL is reported as endemic. Outbreaks from some settled districts (Peshawar, D.I. Khan, Bannu, Hangu, Karak, Nowshera, Dir. upper and lower, Shangla, Dargai,) and FATA (Parachinar, Khyber, Kurram, Teerah, Orazkzai, South Waziristan, and North Waziristan) have been recorded (Mubbashir Hussain et al., 2017). In Pakistan, a total number of 6011 cases of CL were being reported by the health directorate in the ex- FATA from January to 18 April 2018. The cases were reported from six districts: Mohmand, Bajaur, Khyber, North Waziristan Frontier Region, Kohat, and Orakzai (WHO, 2018). The Khyber Pakhtunkhwa province is situated in the north-west of Pakistan and shares a nearly 2300 km extended border (Durand line) with Afghanistan. The distance between Kabul, the capital of Afghanistan, and Peshawar (Khyber Pakhtunkhwa) is 290 km and is thought to have the highest incidence of CL in the world, with approximately 67,500-200,000 cases records annually among the inhabitants of 3.7 million individuals (Hussain *et al*., 2017). The occurrence of diseases generally depends upon gender, pointing to behavioral designs that increase vector exposures, house design age, construction material, and the existence of domestic animals in that region. In the northern region of Pakistan, childhood CL is most prevalent (Wasunna et al., 2016). Moreover, the infection spreads to non-endemic areas mainly by the migration of the infected refugees and local residents (Shaheen et al., 2020). Looking at the presence of a dry, hot, and humid climate with an abundance of sand-fly vectors and cross-border movements, the prevalence of CL is high in the Khyber Pakhtunkhwa and ex-FATA at the Pakistan-Afghan border (Hussain *et al*., 2017).

1.3 Life cycle and transmission of *Leishmania*

The life cycle of the *L. tropica* complex is completed in two hosts (Fig 1.2). Man, dog, and other animals are the primary hosts. Female sandfly is the vector (Phlebotomus species). The following sandfly species serve as the vector. *P. intermedius, P. pappatasi, P. causasiasus, and P. sergenti.* Transmission strategy: The most typical way to acquire the disease is by a sandfly bite. Direct touch can also occasionally result in infection. Direct inoculation of amastigotes can cause infection to spread from person to person or animal to person. Autoinoculation is another method of infection. The incubation period might last anywhere between 2 and 8 months (CK Paniker, 2007).

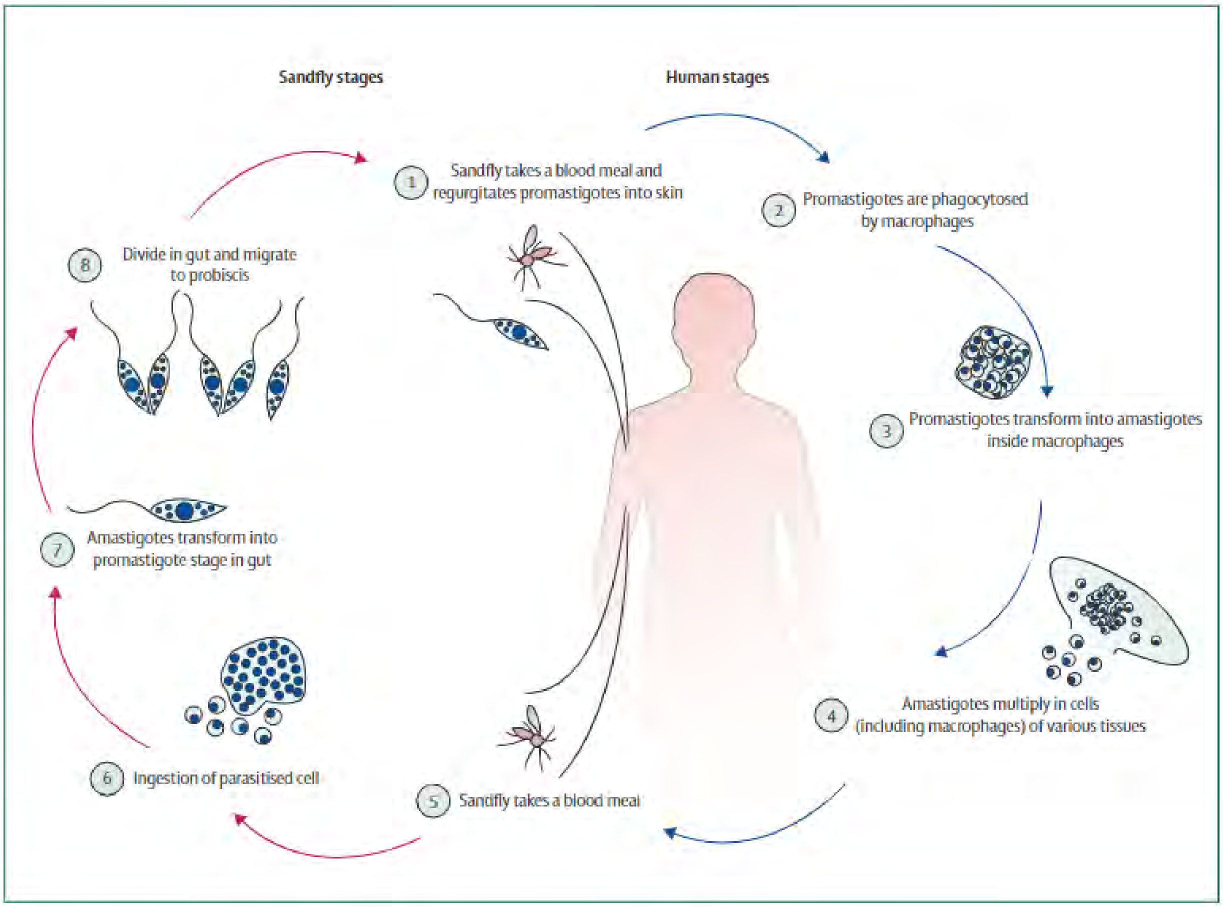

Figure 1.2: A complete life of Leishmaniasis (Reithinger et al., 2007).

The proboscis-induced lesion on the sandfly causes the promastigotes to disgorge. Promastigote is an infectious form and is found in the midgut of female sandflies. Infected female sandflies bite humans, which spreads the disease. The sandfly spills again. The reticuloendothelial system cells (macrophages, polymorphonuclear leucocytes and monocytes) ingest them and transform them into amastigote (LD bodies) inside the cells. The amastigote divides by binary fission, creating a large number of daughter cells that enlarge and rupture the macrophage. Additionally, histiocytes and macrophages phagocytose the liberated daughter cells. In neutrophils or monocytes, a limited number of LD bodies could be observed in peripheral blood. The amastigotes found in tissue fluids and peripheral blood enter the vector sandfly alongside its blood meal when it feeds on an infested human. The amastigote lengthens and transforms into the promastigote stage in the sandfly's midgut (stomach). By longitudinal binary fission, the promastigote multiplies and grows to huge sizes. They could be seen as massive rosettes with tangled flagella. In sandfly, they travel through the midgut towards the pharynx and then hypostome, where they assemble and obstruct the passage. These obstructed sandflies have trouble sucking blood. Plugs of adhering parasites may be dislodged from the pharynx and dropped into the perforated hole when they bite a person and try to suck blood. After ingesting the amastigotes, it takes the promastigotes around 10 days to multiply enough to obstruct the sandfly's throat and oral cavity. Therefore, this is the length of the extrinsic incubation time. Additionally, this period coincides with the vector's gonadotropic cycle such that amastigotes taken during a particular blood meal are prepared for transmission when the sandfly consumes a blood meal after laying its eggs. The epidermis, big mononuclear cells, neutrophils, capillary endothelial cells, as well as free amastigotes in the tissues all include amastigotes. Sandflies that are eating close to the skin lesions consume them. The amastigotes transform into promastigotes in the sandfly's midgut, where they multiply rapidly. After being phagocytosed via mononuclear cells, the promastigotes are then transferred to the skin of individuals bitten by sandflies, where they proliferate and form amastigotes. However, unlike in visceral Leishmaniasis, they remain on the skin and do not spread to the internal organs. (Paniker *et al*., 2017)

1.4 Pathology

The endothelial cells and histiocytes both have amastigote forms. The plasma cell and lymphocyte infiltration is seen along with an inflammatory, granulomatous response. Early lesions start out as papules and progress to ulceration necrosis. The two most common pathological lesions are papule and ulcer. They leave scars as they recover over months to years. Compared to *L. tropica, L. major* causes more quickly healing wounds. Lowland regions of Asia, Africa and the Middle East, exhibit this. The reservoirs are rodents like rats, gerbils, and others. Most significantly, *P. papatasi* is indeed the vector. The non-ulcerating, frequently diffuse lesions produced by *L. aethiopica* and observed in the Ethiopian highlands and Kenya and is called as Diffuse-cutaneous Leishmaniasis. The typical vector is *P. longipes*. Even though the nodular lesions are only seen on the skin, it is an uncommon type of illness. Low humoral in addition to cell-mediated immunity are its defining characteristics. The lesions might persist a lifetime or even for years. It is challenging to treat. (Paniker *et al*., 2017)

1.5 Laboratory Diagnosis

A smear is prepared from material that has been Giemsa- or Leishman-stained and is taken from the indurated margin of a nodule or sore. The macrophages contain a considerable quantity of amastigotes. By demonstrating amastigote in the smear taken from the lesion, a definitive diagnosis is achieved (Paniker *et al*., 2017). By cultivating the aspirate material in NNN medium, promastigote forms may be separated (Paniker *et al*., 2017). This is a useful skin test. Children under 10 from endemic regions who have a positive leishmanin test are highly predictive of the infection. In cases of diffuse cutaneous Leishmaniasis, the skin test is negative (Paniker *et al*., 2017).

Serology results have little significance because the patient has no measurable levels of circulating antibodies (Paniker *et al*., 2017). Control and prevention programs depend directly on targeting the exact species of the parasites. However, the species of *Leishmania* are morphologically and physically similar and cause different forms of CL, so it is impossible to differentiate between species alone by microscopy (Hosseini Farash et al., 2020). The diagnosis of CL can be made clinically, however, to distinguish the infection from other skin diseases, parasites are identified by direct examinations of their amastigotes, by polymerase chain reaction or culture in a specific media (Arif et al., 2022). The identification of *Leishmania* parasites is essential not only from an epidemiological point of view but also on clinical ground too. In order to choose diagnostic procedures, define patient prognosis, treatment plan and monitor clinical results (Akhoundi et al., 2016).

There are various therapies or treatment being used for different forms of Leishmaniasis, and the agent of choice is the systematic use of pentavalent antimony from the last decade, which is present in meglumine antimonite. These agents have several toxicities and is growing ineffective due to parasite resistance (Mcgwire & Satoskar, 2014). Due to the lack of a vaccine, pentavalent antimonial drug treatment is still the first line of treatment for Leishmaniasis in Pakistan, as in other parts of the world. The most important drawback associated with the treatment of Leishmaniasis is that, it is financially costly and painful (Firdous et al., 2009). The increase in Leishmaniasis prevalence is mainly due to several factors; including the lack of an effective vaccine, the AIDS epidemic, increased international conflicts, and emerging resistance to chemotherapy. For control of the disease different approaches and methods have been used, including vector control strategies and vaccination (Ong et al., 2020). Science-based vaccination for most *Leishmania* species is not yet available and control of the disease depends solely on chemotherapy (Pourmohammadi et al., 2011).In the clinical laboratories of most rural areas, microscopy is being used for regular diagnosis, creating an opportunity for non-precise diagnosis, which can affect the health of patient and the health care system. In addition, false positive reports may result in unnecessary treatment with possibly toxic drugs. Similarly, false negative reports can lead to a persistent disease course along with greater patient suffering (Lamm et al., 2018). Despite of the afore-mentioned drawbacks, parasitic cultivation and microscopic identification still remain the main diagnostic tools for Leishmaniasis being used in most endemic areas. However, using either technique, accurate and rapid species identification is not yet possible (Bettaieb et al., 2020).

An occasional *Leishmania* skin test (LST), or Montenegro test, is the easiest and most reliable source of diagnosis in the field studies of present or past infection of exposed *Leishmania* parasites (Carstens-Kass et al., 2021). In recent years, the introduction of polymerase chain reaction (PCR) analysis has been proven to be the most suitable and accurate molecular tool for direct detection and characterization of *Leishmania* parasite species in clinical samples (Yehia *et al.,* 2012). PCR-based analysis is more accurate and sensitive than microcopy or culture. Histological observation is the most frequent Leishmaniasis diagnostic test in which a tissue imprints, a droplet or smear of tissue is applied to a glass slide, stained with Giemsa, and examined using oil emersion lenses. The tiny amastigote form appears as round or oval bodies ranging from 2-4 µm in diameter with a characteristic nucleus and rod-shaped kinetoplasts (Barkati et al., 2019; de Vries et al., 2015). The diagnosis of cutaneous Leishmaniasis requires a clinical examination in conjunction with parasitological molecular approaches (van den Bogaart et al., 2014). The parasitological procedures, which are regarded as the gold standard method, encounter certain challenges since the lesion has a low parasite count as the parasite only located on the skin. While molecular approaches offer the benefits of high sensitivity and specificity traditional methods, like direct culture, were time-consuming and can only be carried out in particular facilities with expert microscopists (Younis et al., 2021). Standard DNA extraction methods, however, are costly, time-consuming, and probably bear the chance of unexpected cross-contamination throughout their several processes (Goonoo et al., 2022). Therefore, a simple method is needed to isolate DNA from pigmented smears. Conventional FTA cards (Whatman filter paper) appear to be acceptable for these objectives as well as for long-term preservation and maintenance of DNA without the need to store them in a freezer, according to prior investigations (Izadi et al., 2016).

To the best of our knowledge, there are no records of previous investigations and no more information available regarding the precise prevalence of *Leishmania* species, including any work on the identification of CL based on spatiotemporal analysis of the concerned region i.e D.I.Khan and adjacent areas. Cutaneous Leishmaniasis is considered the major threat to the public in the northern region of Khyber Pakhtunkhwa. Limited studies were conducted due to a lack of health resources, financial resources and appropriate knowledge. Therefore, this study was designed to determine the prevalence of *Leishmania* species from CL suspected patients in the district D.I.Khan, Khyber Pakhtunkhwa, Pakistan.

1.6 Objectives

The objectives of the present study were:

1. To find the prevalence and most associated risk factors of cutaneous leishmaniasis in D.I. Khan district of Khyber Pakhtunkhwa, Pakistan.
2. To investigate the spatiotemporal distribution of cutaneous leishmaniasis in D.I.Khan by using geographic information system (GIS).

MATERIALS AND METHODS

2.1 Study Area

The current research was conducted in Dera Ismail Khan with latitude 31.8626° N, longitude 70.9019° E, located in the province KP. The targeted region for study has a dry, dusty environment with warm summers and moderate winters, and is supposed to be one of the most significant endemic locations for CL. District includes 5 tehsils namely D.I.K, Daraban, Kulachi, Paharpur and Paroa. Data also includes number of patients in adjacent areas lined to the boundaries named Tank, D.G.Khan, Lakki marwat, Mianwali, Bhakkar, Waziristan, Bannu and Karak etc.

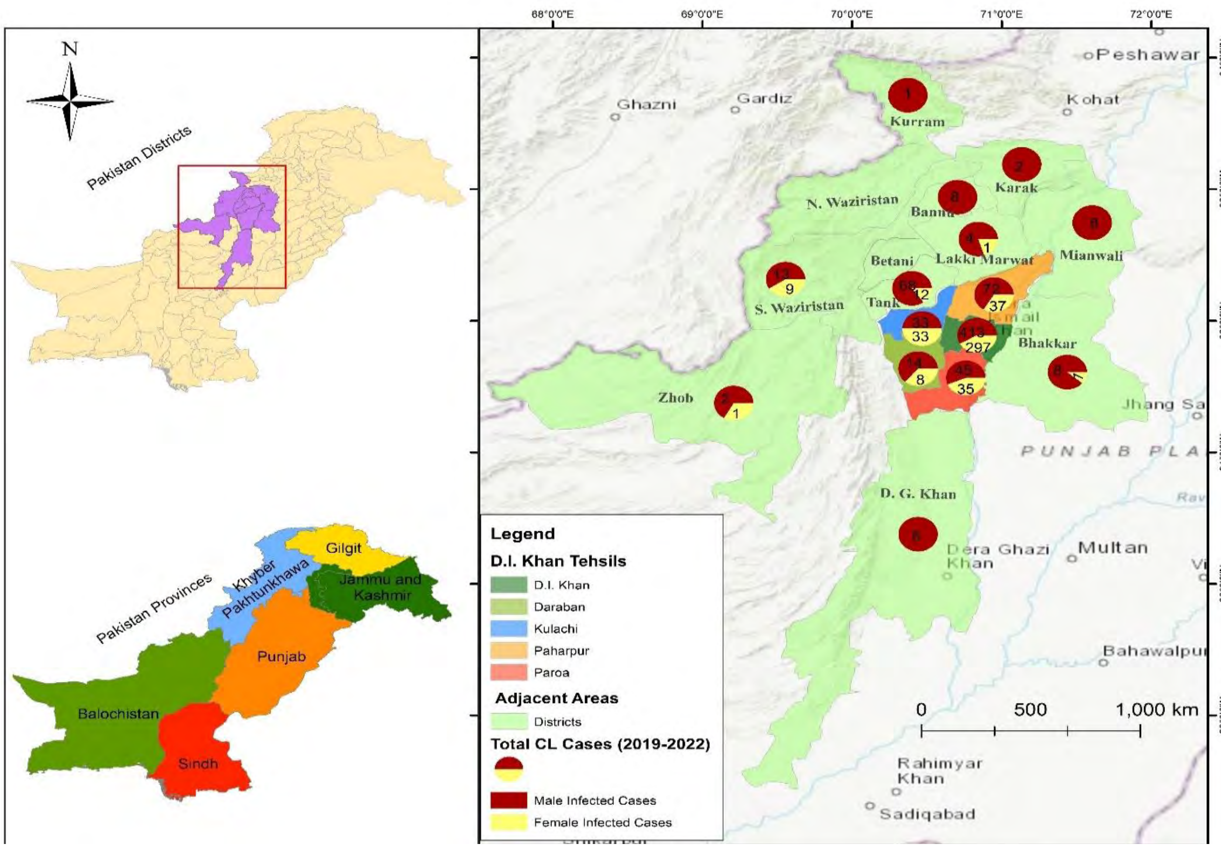

Figure 0.1 : Map of study area including exact location of sampled villages with defined male and female ratio.

2.2 Ethical consideration and consent

The current study was conducted using both primary and secondary data that did not include patient names or personal information. A signed informed consent was acquired along with Ethical Review Board permission for the research. It was stated that the data wouldn't be shared with anybody and would only be retained for study purposes. All procedures were completed in compliance with the applicable laws and regulations.

2.3 Collection of Leishmaniasis health records

A tehsil-wise epidemiological data was collected from different local hospitals and CL centers, i.e. District Headquarter Hospital D.I.Khan and Tehsil Headquarter Hospital Kohat. The study was carried out between January 2019 and March 2022 with the district health officer's consent. There were 1,135 total studied subjects, of whom 987 were from district D.I. Khan and 148 were from nearby locations. Patients of various sexes, socioeconomic statuses, and ages are included in the data. All the patients had one or more lesions on their skin including face, upper extremity and lower extremity.

2.3 Questionnaire and Sample collection

Before addressing the patients, permission was requested from the doctors in the hospital, and after that, either every positive patient or their representatives filled out a questionnaire. A detailed questionnaire was designed which included patient's demographic information such as age groups (1-20, 21-40, 41-60, >61), location of lesion (face, upper extremity, lower extremity), gender (male, female), type of lesion (dry or mucoid), Number of lesion (single, multiple), Duration of lesion (in months), locality, socioeconomic position, literacy and other parameters.

2.4 Laboratory diagnosis and microscopy

Samples were taken for microscopic examination in the supervision of medical technicians. The infected lesion was cleansed and sterilized using an alcohol swab before being penetrated with a sterile lancet to collect the samples. The thumb and fingers were used to press the lancet. Since methanol makes it easier for blood to adhere to slides, it was initially maintained in methanol for the examining slide. To identify the parasite (amastigote or promastigote) , blood from the lesion's scraping region was deposited on both sides of microscope slides . On the same slides, two different blood stains formed. While one kind of smear was created thick, the other kind was made thin. The smears were maintained in 100% methanol for two to three minutes after being air-dried. The slides with thick and thin blood smears were then stained with Giemsa for around 45 to 60 minutes in the staining jar. The slides were carefully stained, and after examining the amastigote and promastigote forms of *Leishmania* spp. under an oil immersion microscope, in the absence of amastigote within or outside the macrophage determined the slides to be negative (Jamal et al., 2013).

2.5 Spatiotemporal Analysis

The annual incidence rate of CL infections across the study area was calculated. The geographical coordinates of the patients' residences were collected using the software version (7.3.0) of Google Earth. After assortment, CL cases and climatic data were analyzed through ArcGIS version 10.8 and spatial analysis (Ali-Akbarpour et al., 2012; Gomez-Barroso et al., 2015).

Data obtained from filled questionnaires was added to Microsoft excel windows 2013 version and arranged by different variables. For further analysis, the socioeconomic coordinates of given areas were determined using Google Earth Pro (version 7.3) and compared to the corresponding CL average incidence. In order to improve the model's prediction ability, spatial analysis and using ArcGIS, geographical risk analysis was carried out in version 10.8. Additionally, the proportionally symbolized, extracted DEM map and obtained data were used to determine the geographic risk of CL infection, and Zonal Statistics was then used to the DEM output data to determine the real elevation of each afflicted tehsil and hamlet.

2.5.1 IDW spatial interpolation, choropleth and focal statistics

A choropleth map was created using four categories of metrics to demonstrate the lack of uniformity amongst tehsils (area-wise, age-wise, altitude and range-wise). For model accuracy, field statistics were employed (Pakzad et al., 2017). The CL focal transmission rate was assessed by using Inverse density weight (IDW) tool from the interpolation group of geostatistical tools. This is a complex and dynamic estimating strategy where readings at unsampled sites are decided by a sequential set of variables at known sampled spots, supposing a local influence that diminishes with distance (Pigott et al., 2014). To ascertain the focal transmission of CL incidence in the closest and farthest zones, focal statistics with a circular pattern were chosen, and the obtained IDW mean values were used (Zeb et al., 2021).

2.5.2 Cluster and outlier analysis

The Spatial statistics tools set was implemented for the Cluster and outlier analysis (Anselin Local Moran I) of the biggest hot spots at the tehsil or village level. The cluster and outlier analyses reveal five distinct geographic classes. Areas where a location's value differs significantly from that of its neighbours, either significantly higher or lower, and unusual locations where a location's value is high or low in relation to its surroundings(Pakzad et al., 2017)

2.6 Statistical analysis

Used SPSS software (version 21, IBM Inc., USA) to analyze the data. One way ANOVA test was performed with a confidence interval of 95% to find out possible relationship between the incidence or prevalence of CL disease and variables like gender, age, lesion site, lesion number, onset of lesion and Tehsil or locality. Considered the standard $p<0.005$ value as statistically significant. Designed graphs in GraphPad prism version 5.50.

RESULTS

A total of 1135 medically diagnosed CL patients were recorded over the study's time frame. Cutaneous lesions, ulcers, and nodules have been identified as the most frequent clinical characteristics among studied participants. Figure 3.1 a,b and c represents the early and late lesions of cutaneous leishmaniasis on different body parts.

Figure 3.1 (a): Early onset of CL in patients at DHQ D.I.Khan.

Figure 3.1 (b) and (c): Late onset of CL in patients at DHQ D.I.Khan.

Geimsa-stained needle evacuated smears revealed amastigotes of *Leishmania* in all patients (100%) and validated the laboratory diagnosis (Figure 3.2).

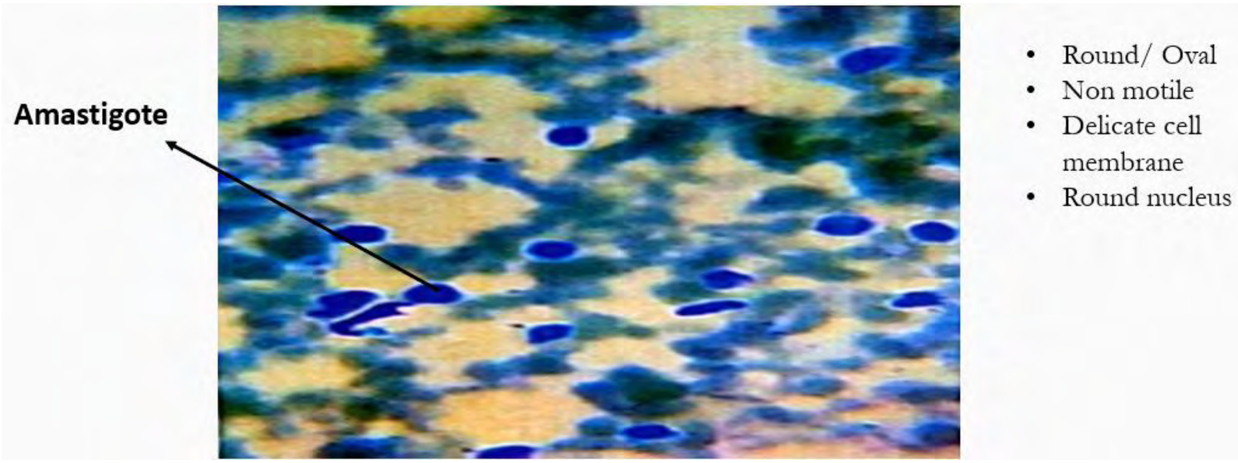

Figure 3.2: Representing the amastigotes under microscope.

3.1 Age wise distribution of CL

The epidemiological data revealed that CL was widespread in people of all ages. In comparison to other age groups, the age group 1-20 years had the largest age-wise distribution, with n=734(65%). The lowest percentage of CL cases were found in the age groups 21–40 and 41–60, with n=274 (24.1%) and 101 (9%), respectively. The age group over 61 had n=26 (2.3%), showing that a significant reduction in number of cases (Figure 3.3). However, CL cases had significant (R^2= 0.64; P=0.05), association with different age groups (Table 3.1).

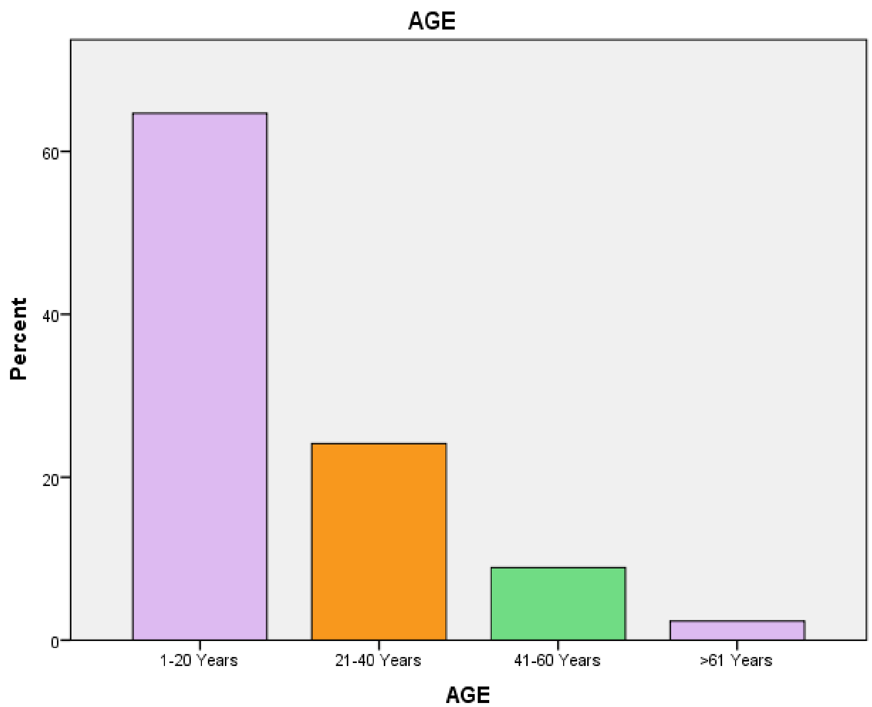

Figure 3.3: Number of CL cases in different age groups.

Table 3.1: Age-wise association of CL cases among study participants.

Variables		2019 n (%)	2020 n (%)	2021 n (%)	2022 n (%)	Total (%)	R(adjusted)	R(squared)	Significant value
Age groups (years)	1-20	262(70)	271(65)	146(68)	55(71)	734(64.7)	0.556	0.645	0.005
	21-40	114(27)	95(23)	46(21.3)	19(24.3)	274(24.1)			
	41-60	38(9)	41(10)	66(31)	2(3)	101(8.9)			
	≥61	9(2.1)	12(3)	3(1.3)	2(3)	26(2.3)			

3.2 Gender wise distribution of CL

The number of male patients was large n=700(61.7%), whereas the number of female patients was lower n=435(38.3%) in 4 years of study (Fig. 3.4). However, statistically no significant (R^2= 0.16; P=0.316), association was observed between leishmaniasis and gender, which means there is almost equal rate of infection for both sexes (Table 3.2).

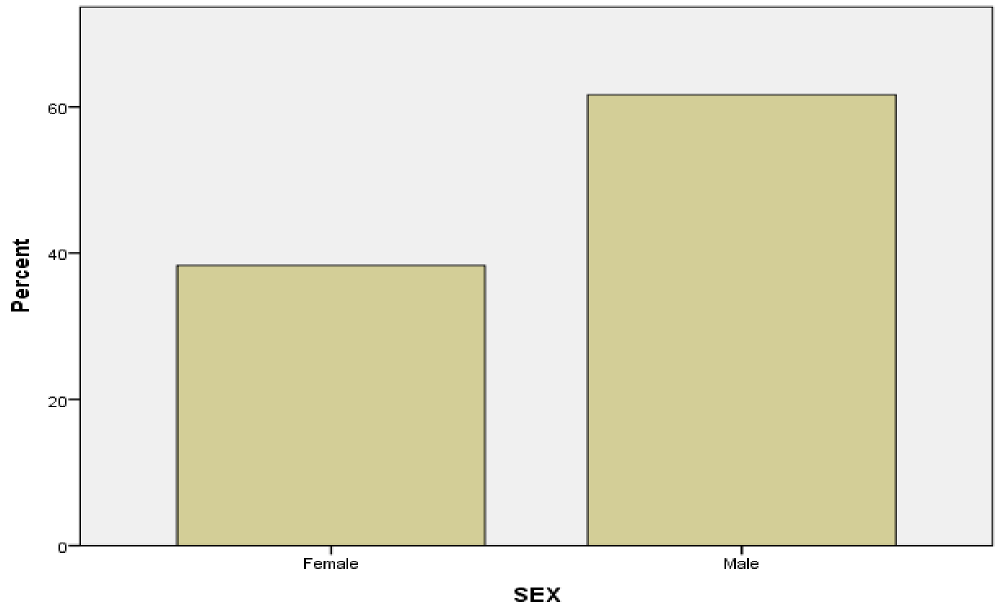

Figure 3.4: Gender wise number of CL cases.

Table 3.2: Gender-wise association of annual distribution of CL cases.

Variables		2019 n (%)	2020 n (%)	2021 n (%)	2022 n (%)	Total (%)	R(adjusted)	R(squared)	Significant value
Gender	male	256(61)	259(62)	132(61.3)	53(68)	700(61.7)	0.27	0.166	0.316
	female	167(39.4)	160(38.1)	83(39)	25(32)	435(38.3)			

3.3 Area wise distribution of CL

Large number of the patients (n=989) in our research were from densely populated, undeveloped regions with substandard housing and slum areas. Most of which were from lower socioeconomic classes. According to our studies CL was found to be prevalent in all 5 tehsils of D.I.Khan as well as in the areas of adjacent boundaries. Many cases were found in tehsil D.I.K n=712(62.7%) with a decline number of cases in other 4 tehsils, n=109(9.6%) in Paharpur, n=80(7%) in Paroa, n=66(5.8%) in Kulachi and n=22(1.9%) in Daraban which is lowest number of cases. The adjacent areas including Tank, D.G.Khan, Lakki marwat, Mianwali, Bhakkar, Waziristan, Bannu and Karak etc contributed the 12.9% of leishmaniasis cases with n=146 (Fig.35). However, the statistically significant (R^2= 0.66; P= 0.001), association was recorded between disease and studied districts

(Table 3.3). Map showing the area-wise male female ratio of CL cases across the five district and nearby areas of D.I.K (Fig.3.6).

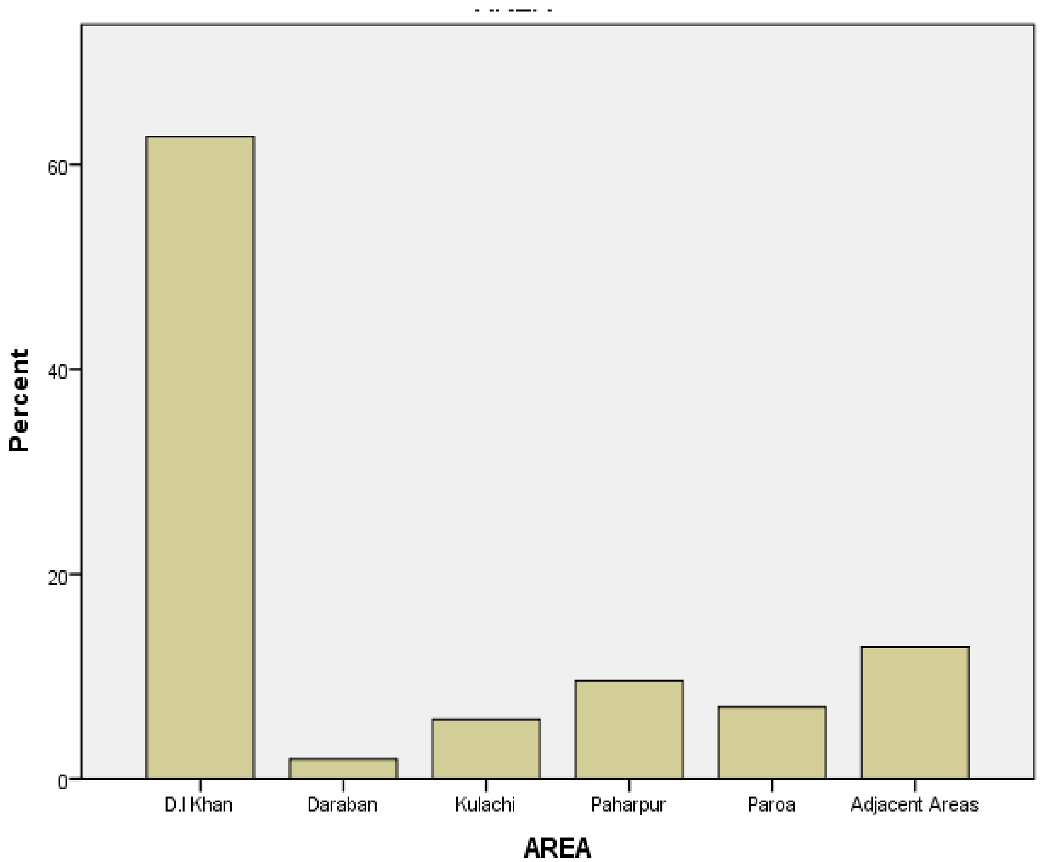

Figure 3.5: Area-wise number of CL cases in study participants.

Table 3.3: Area-wise association of CL cases among study participants.

Variables		2019 n (%)	2020 n (%)	2021 n (%)	2022 n (%)	Total (%)	R(adjusted)	R(squared)	Significant value
Area	D.I.K	286(68)	256(61)	129(60)	41(53)	712(62.7)	0.57	0.663	0.001
	Daraban	6(1.4)	15(4)	1(0.4)	0(0)	22(1.9)			
	Kulachi	13(3)	43(10.2)	8(4)	2(3)	66(5.8)			
	Paharpur	26(6.1)	29(7)	40(19)	14(18)	109(9.6)			
	Paroa	26(6.1)	23(5.4)	19(9)	12(15.3)	80(7)			
	Adjacent areas	66(16)	53(13)	18(8.3)	9(12)	146(12.9)			

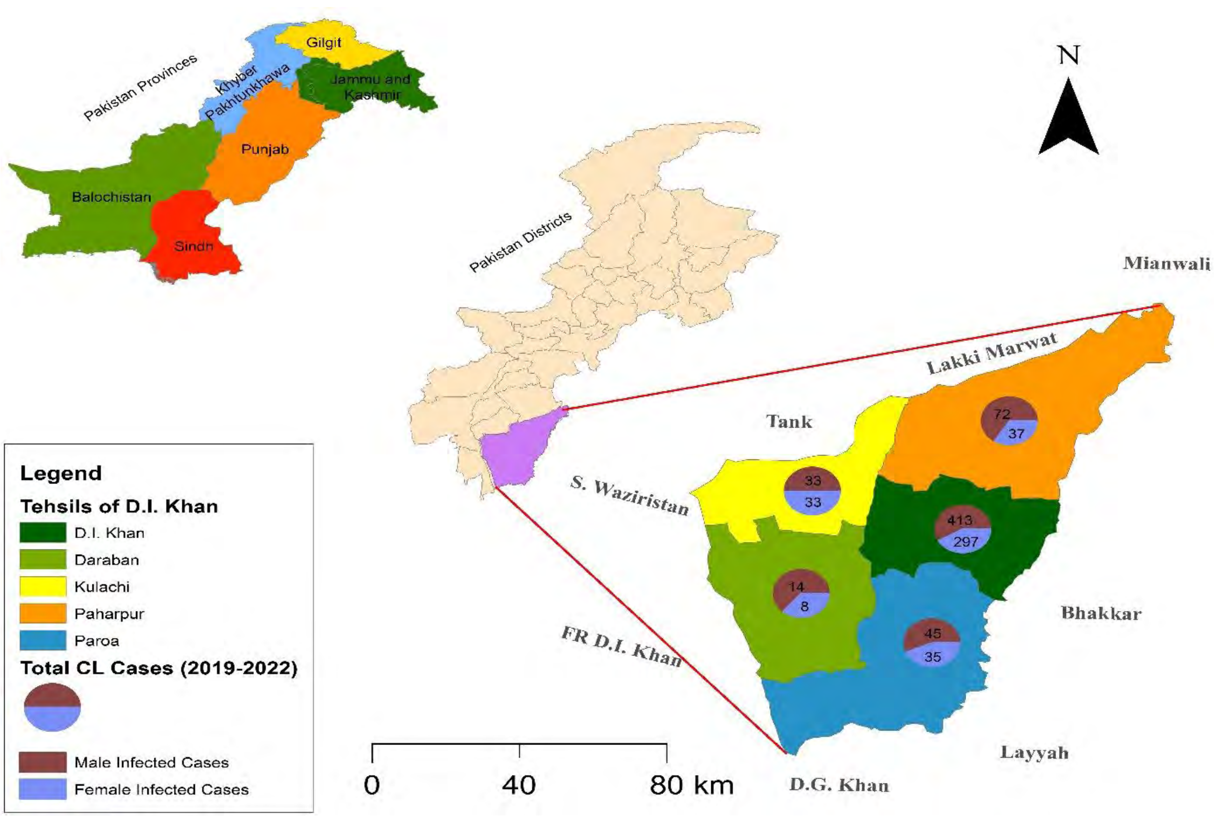

Figure 3.6: Map showing the area-wise male female ratio of CL cases across the district and nearby areas.

3.4 Duration of lesion in months

The interval between the beginning of the CL lesion and the diagnosis might range from less than two months to more than a year. 12–14 weeks after the appearance of the lesion, on average, the patient visited the hospital for the first time. All patients (100%) had early and late lesions, which were categorized as having been present for less than six months and more than six months, respectively (Table 3.4). The lesion duration between 1-2 months was 57.1% (n=648), 3-4 months 24% (n=272), 4-5 months 7.3% (n=83), 7-8 months 7.8% (n=89) and greater than 9 months up to a year and half was 3.8% (n=43) in study period (Fig. 3.7). The result showed statistically significant (R^2= 0.68; P=0.005), reduction in number of lesions with an increase in months (Table 3.4).

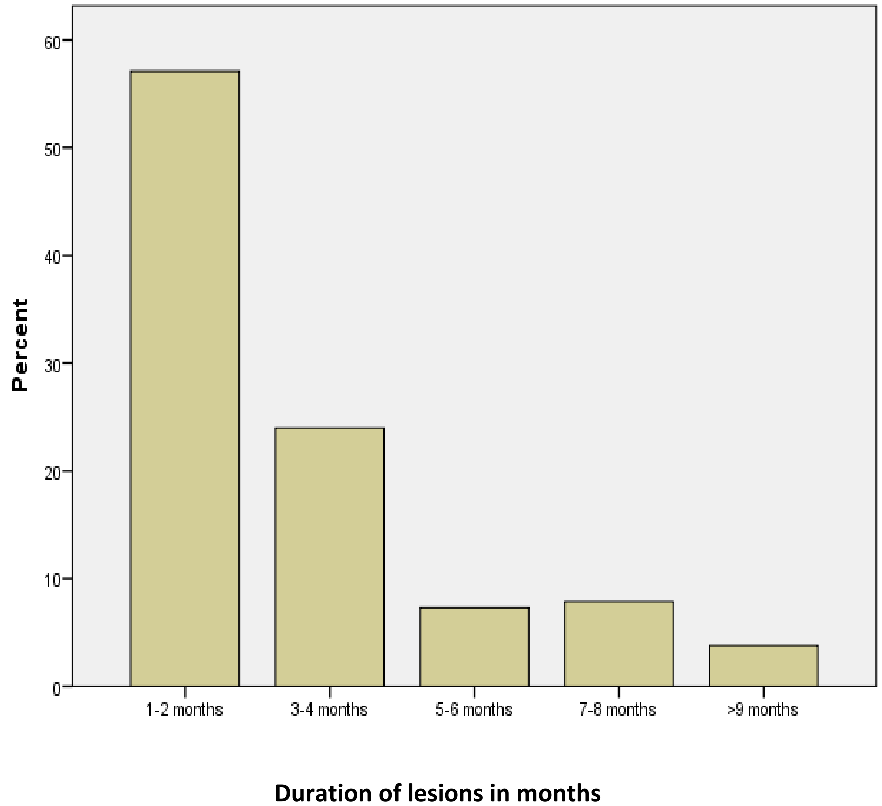

Figure 3.7: Duration of CL lesions in months.

Table 3.4: Association of leishmaniasis with duration of lesion in months.

Variables		2019 n (%)	2020 n (%)	2021 n (%)	2022 n (%)	Total (%)	R(adjusted)	R(squared)	Significant value
Duration of lesion (months)	1-2	239(57)	267(64)	106(49.3)	36(46.1)	648(57.1)	0.503	0.68	0.005
	3-4	100(24)	93(22.1)	56(26)	23(29.4)	272(24)			
	5-6	31(7.3)	15(4)	25(12)	13(17)	83(7.3)			
	7-8	38(9)	29(7)	16(7.4)	6(8)	89(7.8)			
	≥9	15(4)	15(4)	13(6)	0(0)	43(3.8)			

3.5 Number of Lesions

There were 1204 lesions in total among the 1135 patients. Only 864 (76.1%) people have a single lesion, while n=271 (23.9%) have multiple lesions (Fig. 3.8). The results were statically (R^2= 0.42; P= 0.080) non-significant (Table 3.5).

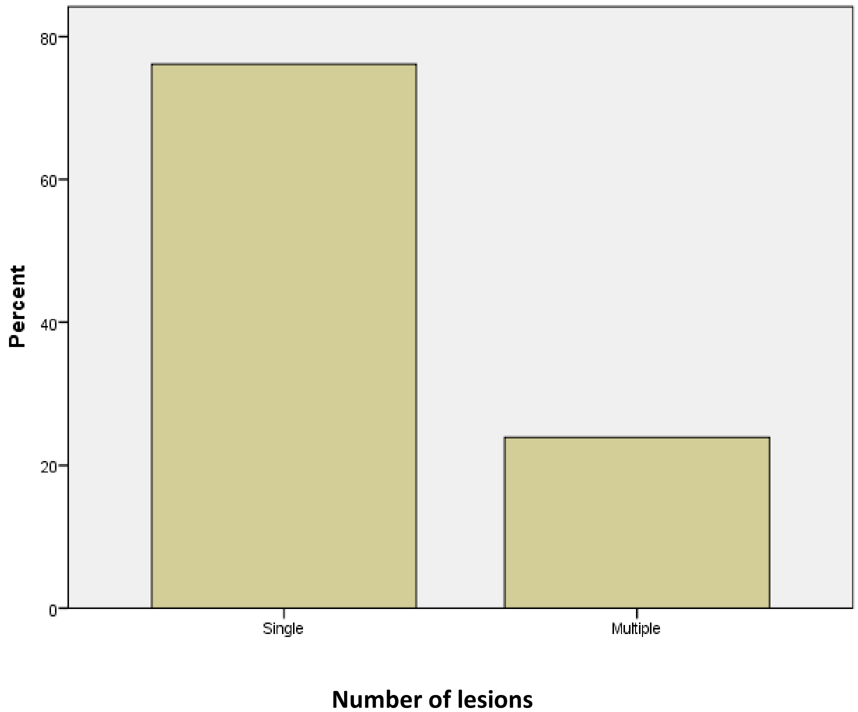

Figure 3.8: Number of lesions identified in CL cases.

Table 3.5: Association of disease with number of the lesions

Variables		2019 n (%)	2020 n (%)	2021 n (%)	2022 n (%)	Total (%)	R(adjusted)	R(squared)	Significant value
No. of lesions	single	316(75)	341(81.3)	154(72)	53(68)	864(76.1)	0.329	0.425	0.08
	multiple	107(25.2)	78(19)	61(28.3)	25(32)	271(23.9)			

3.6 Site of lesions

Most of the lesions were seen on exposed body areas such the face was 30.7% (n=348), upper extremity 29.1% (n=330), lower extremity 34.2% (n=388) and mixed lesions was 6.1% (n=69) (Fig. 3.9). The results showed no signification (R^2= 0.23; P=0.9) association with site of lesions (Table 3.6).

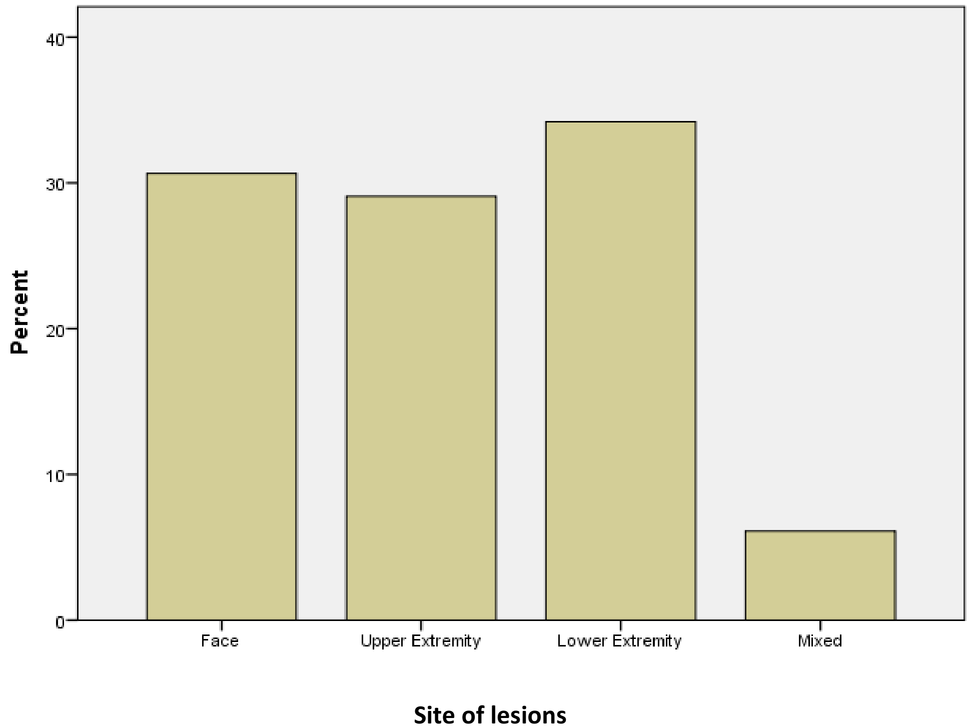

Figure 3.9: The sites of lesions on body among number of CL cases.

Table 3.6: Association with Site of lesions in study period.

Variables		2019 n (%)	2020 n (%)	2021 n (%)	2022 n (%)	Total (%)	R(adjusted)	R(squared)	Significant value
Site of lesions	face	126(30)	128(31)	69(32)	25(32)	348(30.7)	0.194	0.23	0.9
	upper extremity	116(27.4)	107(26)	69(32)	38(49)	330(29.1)			
	lower extremity	142(34)	154(37)	77(36)	15(19.2)	388(34.2)			
	mixed	39(9.2)	30(7.1)	0(0)	0(0)	69(6.1)			

3.7 Spatiotemporal Analysis

3.7.1 Spatial prevalence map

Total 989 CL instances, scattered throughout all villages in the district from 2019 to 2022, were detected when the temperature and geographical aspects were taken into consideration. The tehsil D.I.K. has the greatest spatial prevalence of CL infection, followed by Paharpur, Paroa, Kulachi and Daraban (Fig 3.10 A). The high temperature range in these settlements increases the infected sandfly's tendency to bite. The nearby boundaries similarly had the lowest number of CL cases recorded. The high prevalence of CL in district D.I.Khan may be related to the possibility of cross-border travel. A certain drop in temperature can interfere with the vector's capacity to thrive, that is why protection is necessary since the prevalence of CL infection at the lowest ratio of temperature resulted in minimal CL cases. A specific drop in temperature might interfere with the capacity of the vector to live; hence, preventing sandfly bite lowers the CL cases. The prevalence of CL infection in the minimal ratio of temperature resulted in no CL cases.

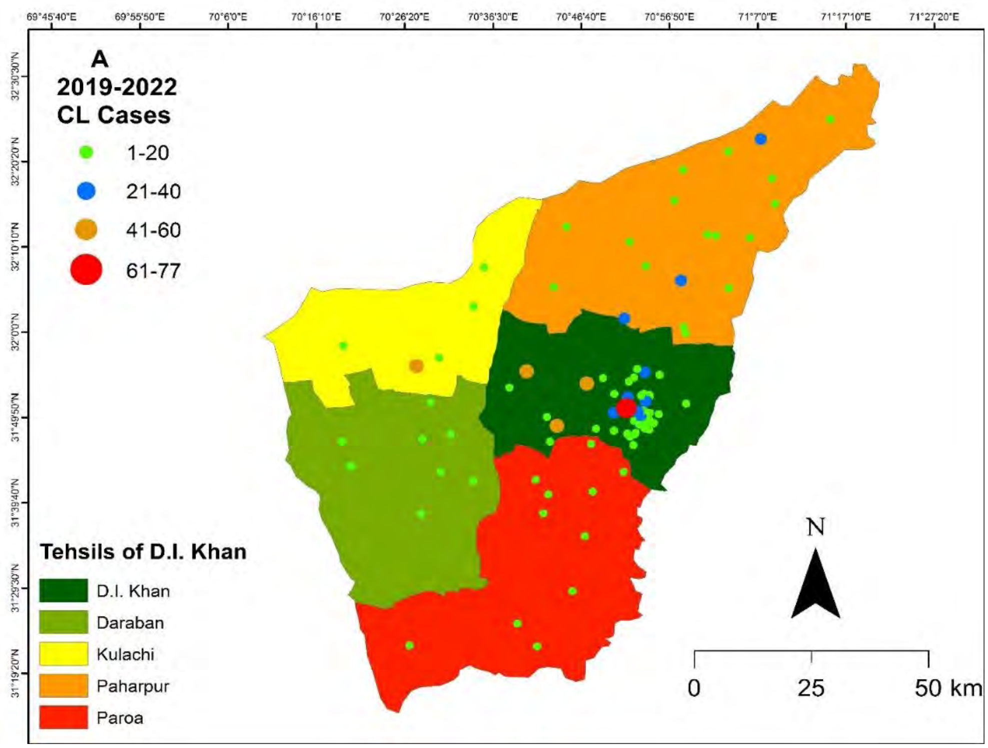

Figure 3.10 A: Represents age-wise highest and least CL incidences in the district D.I.Khan with accordance to villages.

3.7.2 Choropleth map

Map representing the district's highest and lowest concentrations of CL cases. Geographically, a choropleth map showed that Tehsil-wise increasing incidence of CL from Daraban 1-22 cases (2%), Kulachi 23-66 cases (6%), Paroa with 67-80 cases (7%), Paharpur with 81-109 cases (10%) having the lowest CL frequencies followed by D.I.K with reported cases from 110-710 patients (63%) had the greatest CL prevalence(Fig 3.10 B).

Choropleth maps allow us to analyze, contrast and correlate data from multiple sources as well as display geographic information. Color is used in choropleth maps to demonstrate how data is transmitted from one location to the next.

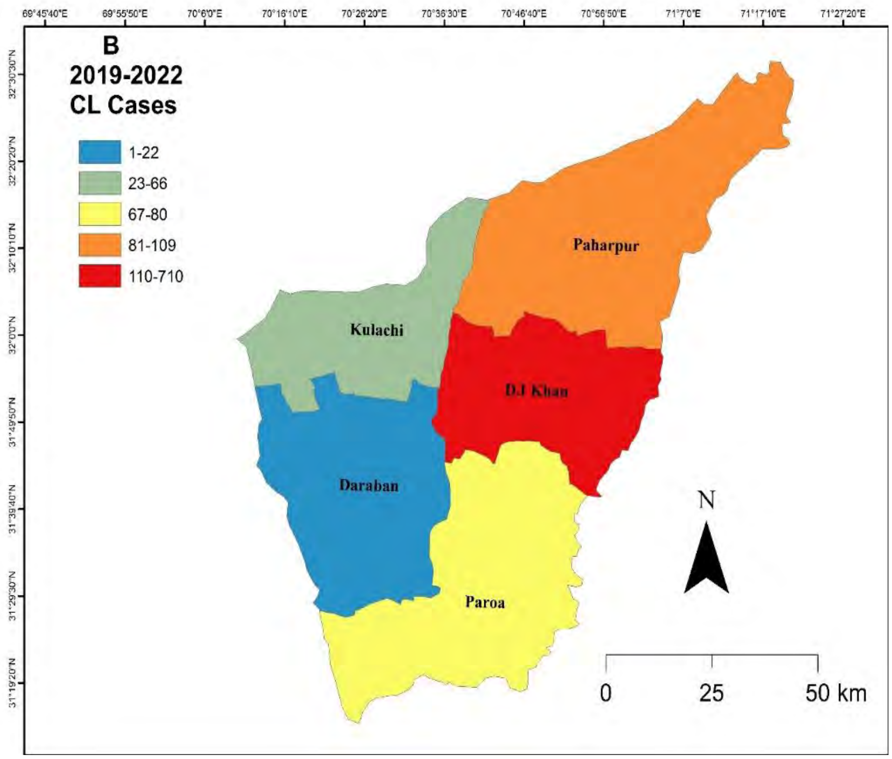

Figure 3.10 B: Choropleth map displaying the greatest to least CL occurrences based on tehsils.

3.7.3 Digital Elevation Model (DEM) map

CL average incidence on the extracted DEM map revealed the presence of CL cases throughout the District Dera Ismail Khan, ranging from 0 to 1172 m. The incidence of CL has been decreased with high altitudes and highest in Zaffar Abad and Chahkan village of Tehsil D.I.K (Fig. 3.10). Each village's geographical coordinates were thoroughly examined, and the highest CL incidences within a range of 0 to 178 m elevations were specified (Table 3.7). High altitudes have a lower prevalence of CL including the areas half of Paharpur and kulachi, and cases more prevalent in the central areas with the lowest elevations from the sea surface are entire tehsil D.I.K and some part of Kulachi, Paroa and Daraban (Fig. 3.10 C). Zonal Statistics was used to assess each village's geographic coordinates in a systematic way and the CL occurrences between an elevation range of

128 to 1365 meters were identified. Dots with different colors represents the age-wise reported cases in high as well as low elevations.

A DEM map represents the topographic region of the Earth's bare ground, which is free of any trees, buildings, and other surface features. DEMs are created from a variety of sources. DEMs are required for mapping and forecasting topographically affected natural risks and hazards, such as landslides and floods. Finding an appropriate interpolation technique is crucial for producing the best estimates of values at unknowable places.

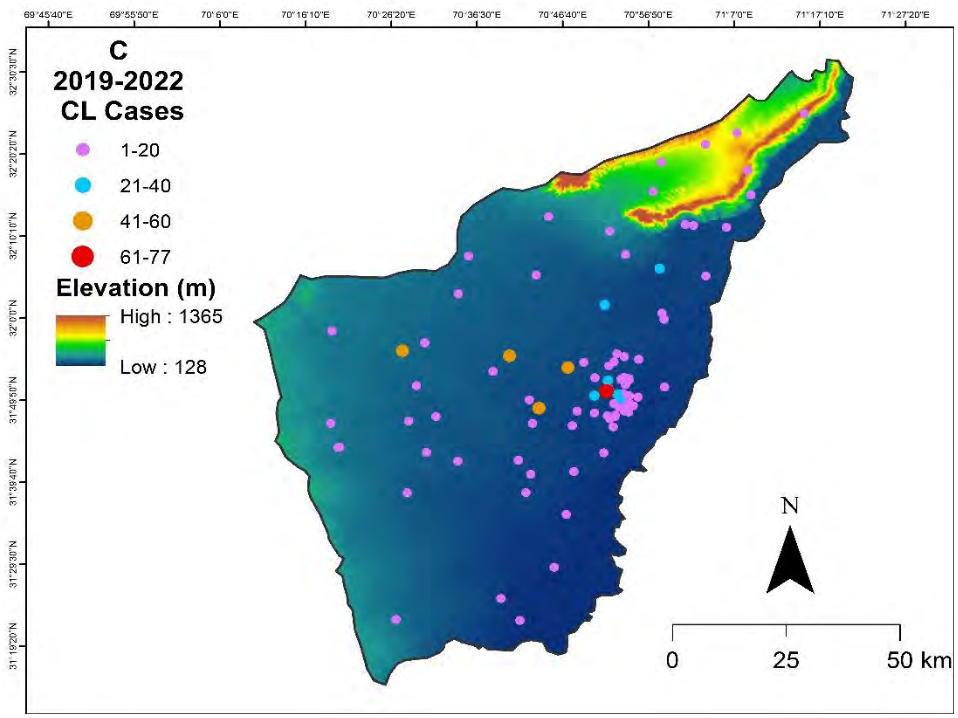

Figure 3.10 C: Map of the average incidence of CL plotted on DEM.

3.7.4 Inverse density weight (IDW) map

The IDW study, which confirms the highest prevalence in urban zones in central regions where the CL hotspots were D.I.K, Kulachi and Paharpur, verified the potential pandemic dangers of CL infection. The research region has high and low endemic areas, according to the IDW analysis's findings. The mean results of the analysis of IDW maps, used to compute the focal distances after careful observation of the transmission patterns (Fig 3.11 A). The future epidemic threats of CL infection were validated by IDW analysis, where the CL cases were high at D.I.K, Kulachi and Paharpur. The map showed CL low and high endemic areas, the focal distances were calculated using the mean values of the IDW analysis after careful observation of the transmission patterns (Fig 3.11 A). The IDW analysis justified the threats of CL infection in the areas located closer to the highest CL presenting villages.

The IDW interpolation method specifically states that entities adjacent to one another are more similar than those farther away. IDW makes advantage of the measured values close by to forecast a value for any unobserved place. CL frequency's focal transmission in the closest and furthest locations, was determined using focal statistics with a circular design and the derived IDW mean values. IDW is a fast, accurate deterministic interpolator. Making judgements on model parameters is not common. It could be an effective approach to get a quick glance at an estimated surface. Although IDW can create "bulls eyes" over data locations, there is no evaluation of prediction mistakes. The data don't need to be predicated on anything. With the use of IDW interpolation, sample points are given weights so that the impact of one spot on another decreases with increasing distances from the new position being estimated.

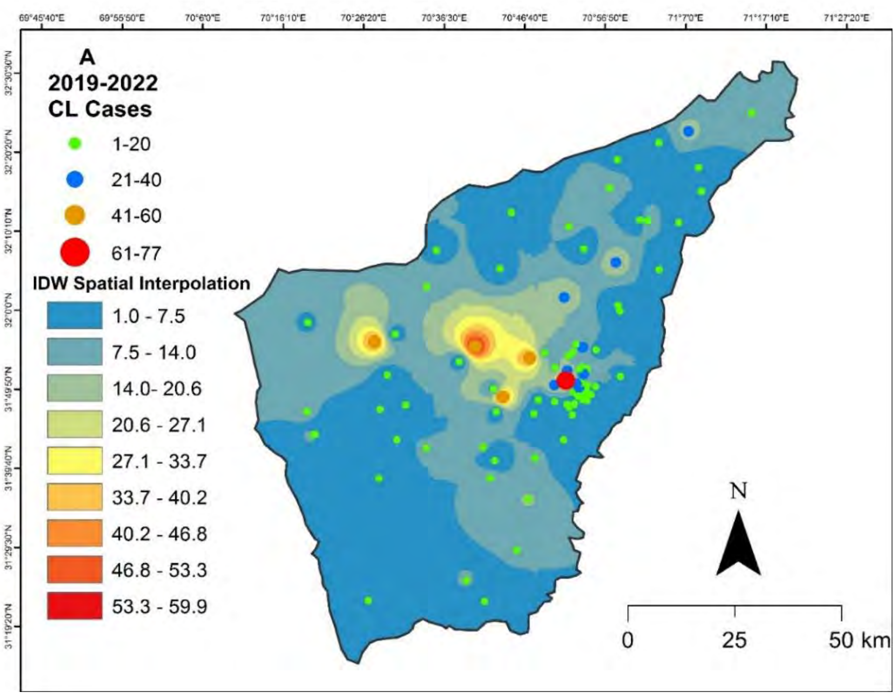

Figure 3.11 A: The average incidence of CL predicted on IDW map for 2019-2022.

IDW mean values (7.5, 14, 20.6, 27.1, 33.7, 40.2, 46.8, 53.3, 59.9) are the predicted values from the given data, software estimated the regions nearby and far away from the concerned area and gave a rough idea about the remaining regions. Red color shows more probability of the cases in estimated area while blue color shows lowest possibility of occurrence of disease in pointed area.

3.7.5 Cluster-Outlier analysis with Focal statistics:

The information supported the likelihood of contracting CL in areas near to villages with the greatest CL prevalence (Fig. 3.11B). Five distinctive geographic groupings are shown by the cluster-outlier analyses. Locations where a spot's value greatly differs from its neighbors' values, whether significantly higher or significantly lower, and unusual regions where a spot's value is high or low in relation to its surroundings. Furthermore, incidents in which no links can be established are referred to be non-significant.

Focal Statistics map uses a neighbourhood operation to generate an output vector whose value is a product of the contents of all input cells in a defined neighbourhood surrounding that point.

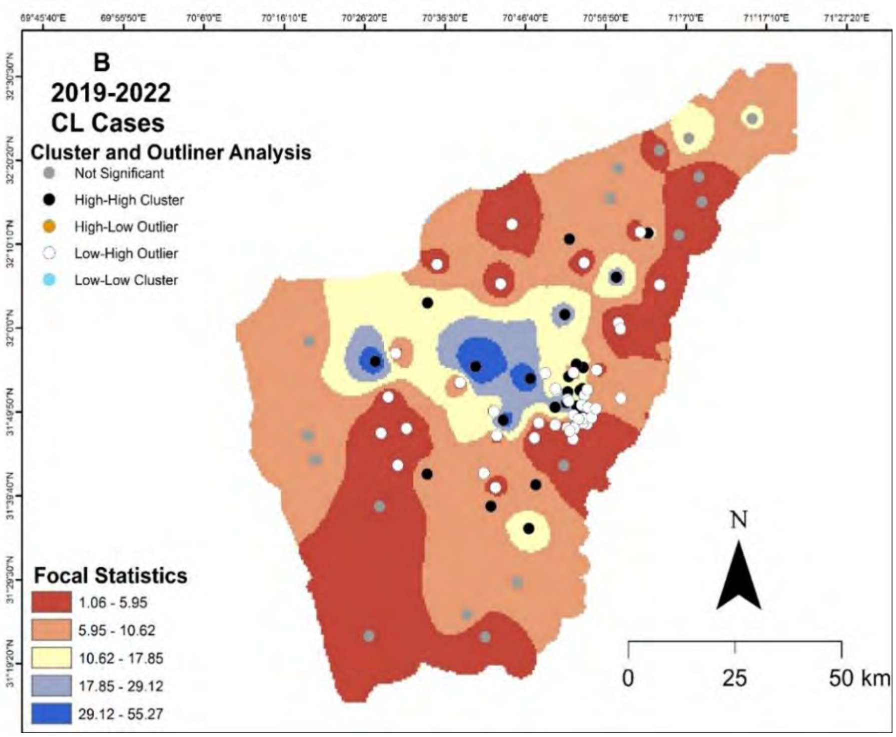

Figure 3.11 B: Cluster-Outlier analysis with Focal statistics.

The high cluster counties according to the Cluster and Outliers study were D.I.K and Paharpur ranging from 42750.0 m to 51300.0 m and Kulachi, Daraban and Paroa ranging from 17100.0 m, 25650.0 m and 34200.0 m respectively, high cluster villages were D.I.K (z-score= 1.8, P=0.071397) and Paharpur (z-score= 1.4, P=0.142513). However, Kulachi (z-score= 1.0, P=0.273971), Daraban (z-score= 1.4, P=0.150079) and Paroa (z-score= 0.9, P=0.318580) were the high and low outlier villages. The other villages were not significant (Fig. 3.11 C).

Clusters covering more area are more significant for the occurrence of disease to the respective regions and vice versa. Software predicts the chances of spread of disease to unknown areas by using the focal statistical tool. The focal values for given map are 5.95, 10.62, 17.85, 29.12, 55.27 represented by different colors red-blue as shown in map. Greater the focal value for a specific spotted region greater are the chances for disease to spread in such region so that we can set the boundaries to such regions from the infected areas or we can take other preventive measures by awaking the people and make them aware about the neglected harmful consequences.

The Mapping Clusters basic tools use cluster analysis to locate statistically important cold spots, hot spots, geographical outliers, and related features or zones. When taking action is required based on the geographical location with one or more clusters, the Map-based Clusters toolset is especially helpful.

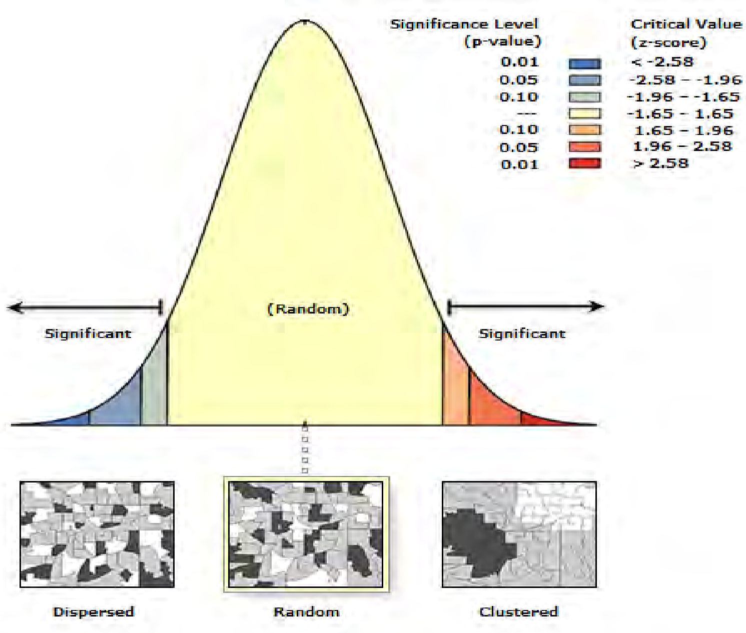

Figure 3.11 C: Graph showing Dispersed, Random and Clustered areas with significant level and critical values evaluated by the software.

Table 3.7: CL Zonal Statistics with elevation (m) of tehsils and high-risk union councils (UC) across the study period (2019-2022).

Variables		Elevation(m)	2019 n	2020 n	2021 n	2022 n	Total cases	R(adjusted)	R(squared)	Significant value
D.I.Khan villages	AbdulKhel	195	4	0	0	0	4	0.47	**0.61**	**0.001**
	AlMuizMill	186	1	0	0	0	1			
	AnjumAbad	160	0	1	1	0	2			
	Arrah	0	3	0	0	0	3			
	Bannu	178	6	2	0	0	8			
	BarkiTown	0	1	1	0	0	2			
	BastiDirkhan	166	1	2	3	1	7			
	BastiSherPao	176	1	0	0	0	1			
	BastiUstrana	178	3	1	0	0	4			
	BayPass	182	6	3	0	0	9			
	Bhakkar	190	5	2	2	0	9			
	Budhani	215	0	1	0	0	1			
	Cantt	210	0	1	0	0	1			
	Chahkan	178	17	22	14	3	60			
	Chandna	179	0	1	0	0	1			
	CRBC chowk	0	3	12	0	0	15			
	DarabanChungi	174	2	1	0	0	3			
	Darazinda	175	0	0	2	0	2			
	Derajaat	172	1	11	10	0	22			
	Dewala	178	1	9	2	2	14			
	Dinpur	0	0	2	0	0	2			
	DiyalRoad	176	5	1	0	0	6			
	FatehMorh	252	10	10	9	0	29			
	FatehPoor	171	2	0	0	0	2			
	GaraAlamKhan	233	0	1	0	0	1			
	GaraJamal	172	0	0	1	0	1			
	GaraMirAlam	175	0	1	0	0	1			
	GilaniTown	0	0	0	1	0	1			
	Giloti	175	0	1	0	0	1			
	Girsaal	0	1	0	0	1	2			
	Gomal	185	6	1	1	0	8			
	GreenTown	177	3	3	0	0	6			
	GridRoad	0	1	0	0	0	1			
	GulshanNisarTown	175	2	4	4	2	12			
	HajiMora	176	0	0	5	0	5			

Hassam	175	0	0	1	0	1
Himmat	181	0	2	0	0	2
IjazAbad	175	0	1	0	0	1
IqbalQilla	180	14	15	0	0	29
KaloKaland	177	0	1	0	0	1
KamraniChowk	173	0	1	0	0	1
Kech	178	8	4	4	6	22
Khanii	0	5	0	0	0	5
Khutti	174	3	0	0	0	3
KiriShamozai	0	1	0	0	0	1
KirriAlazai	0	10	6	0	0	16
Kokar	200	1	0	0	0	1
Korai	207	11	21	10	5	47
KotHabib	214	0	1	0	0	1
KotJai	0	0	1	0	0	1
KotlaSaidan	0	6	5	13	3	27
Lachhra	209	3	15	4	6	28
Lok	0	1	1	0	0	2
MadinaColony	182	4	0	0	0	4
Mandhra	184	0	3	4	0	7
MohallaSariban	0	3	0	0	0	3
MolvianWaliBasti	179	1	0	0	0	1
Mullazai	0	4	2	0	0	6
MuneezAbad	0	4	0	0	0	4
MuqimShah	529	0	1	0	0	1
Muriyali	184	4	11	4	2	21
MusaZaiSharif	0	0	3	0	0	3
Nasir bagh	174	1	0	0	0	1
Nawab	0	0	1	0	0	1
NiaziChowak	0	7	0	0	0	7
NizamPump	183	0	4	0	0	4
Paroa	184	10	5	0	0	15
Police line	178	1	7	5	5	18
Pota	236	0	1	0	0	1
QasooriaTown	0	3	0	0	0	3
QureshiMorh	175	6	2	0	0	8
RattaKulachi	0	2	8	3	2	17
RehmanAbad	0	1	0	0	0	1
SaddarPoliceStation	0	8	2	0	0	10
SaidAbad	0	1	5	5	1	12
SaiduWala	524	2	3	2	8	15

	ShahAlamAbad	0	1	0	0	0	1			
	ShahAlamBurki	0	0	1	0	0	1			
	Shalimar	0	0	1	0	0	1			
	SheikhAbad	173	0	1	0	0	1			
	SheikhYousif	0	1	3	0	0	4			
	ShorKot	182	7	3	2	5	17			
	Siyal	0	4	2	0	0	6			
	WandaKarimShah	0	2	0	0	0	2			
	Yarik	187	0	2	0	0	2			
	ZaffarAbad	0	58	19	0	0	77			
	Zarkani	0	0	1	0	0	1			
Paharpur villages	AwanAbad	180	4	0	3	0	7	**0.08**	**0.23**	**0.09**
	BandKorai	183	1	5	3	3	12			
	BilotSharif	183	2	2	0	0	4			
	DhapShumali	162	0	1	0	0	1			
	GandiUmarKhan	168	0	1	9	0	10			
	KachiMaliKhel	167	1	0	0	0	1			
	KatGarh	173	1	0	0	0	1			
	KattaKhel	175	0	4	0	0	4			
	KirriKhaisore	178	5	4	1	1	11			
	Lar	369	0	1	0	0	1			
	PaharpurTown	161	3	10	8	0	21			
	Paniala	185	3	6	0	0	9			
	Rangpur	181	0	1	2	0	3			
	RehmaniKhel	446	5	0	12	0	17			
	WandaKhanMohammad	204	0	0	5	2	7			
Paroa villages	DarabanKhurd	236	9	1	0	0	10	**0.13**	**0.22**	**0.33**
	LundaSharif	196	2	4	1	1	8			
	Mahra	228	3	4	0	1	8			
	Malana	205	0	1	1	0	2			
	Miran	249	0	0	2	6	8			
	Faqir abad	196	0	1	0	0	1			
	Naivela	178	1	4	3	3	11			
	Ramak	228	2	0	0	0	2			
	Zandani	185	2	17	1	10	30			
Kulachi villages	GaraHayat	183	3	7	2	0	12	**0.05**	**0.36**	**0.25**
	Hathala	217	0	2	2	0	4			
	KotWaliDad	235	1	1	0	0	2			
	Kulachi	221	10	10	10	4	34			
	Looni	212	4	2	1	0	7			
	Maddi	182	2	0	1	0	3			

	Rorri	754	1	1	0	0	2			
	WaliKot	175	1	1	0	0	2			
Daraban villages	DarabanKalan	193	6	1	0	0	7	**0.86**	**0.91**	**0.04**
	GaraEsaKhan	188	0	0	1	0	1			
	KotEssaKhan	769	0	3	0	0	3			
	Saggu	183	8	3	0	0	11			

3.8 Environmental Factors:

When evaluating the effects of environmental factors on CL distribution, we found no evidence that a high incidence of CL was significantly correlated with high temperatures, high relative humidity, or rising annual precipitation.

In our study increased temperature, relative humidity, and precipitation were not significantly associated with CL infection. However previous research has found that warm weather and precipitation help to spread CL because the development of both sandflies and Leishmania accelerates at high temperatures, and precipitation creates more suitable breeding sites for sandflies.

This could be explained by the fact that the time between the onset of the CL lesion and the diagnosis, on average the patient went to the hospital for the first time 12–14 weeks after the lesion initially appeared. The emergence of CL transmission in regions with the lowest temperatures may be caused by movement of non-infected residents to endemic regions.

The climatic data did not show significant association with CL cases in the D.I. Khan district and infection was most prevalent during the winter (Fig. 3.12 ABC).

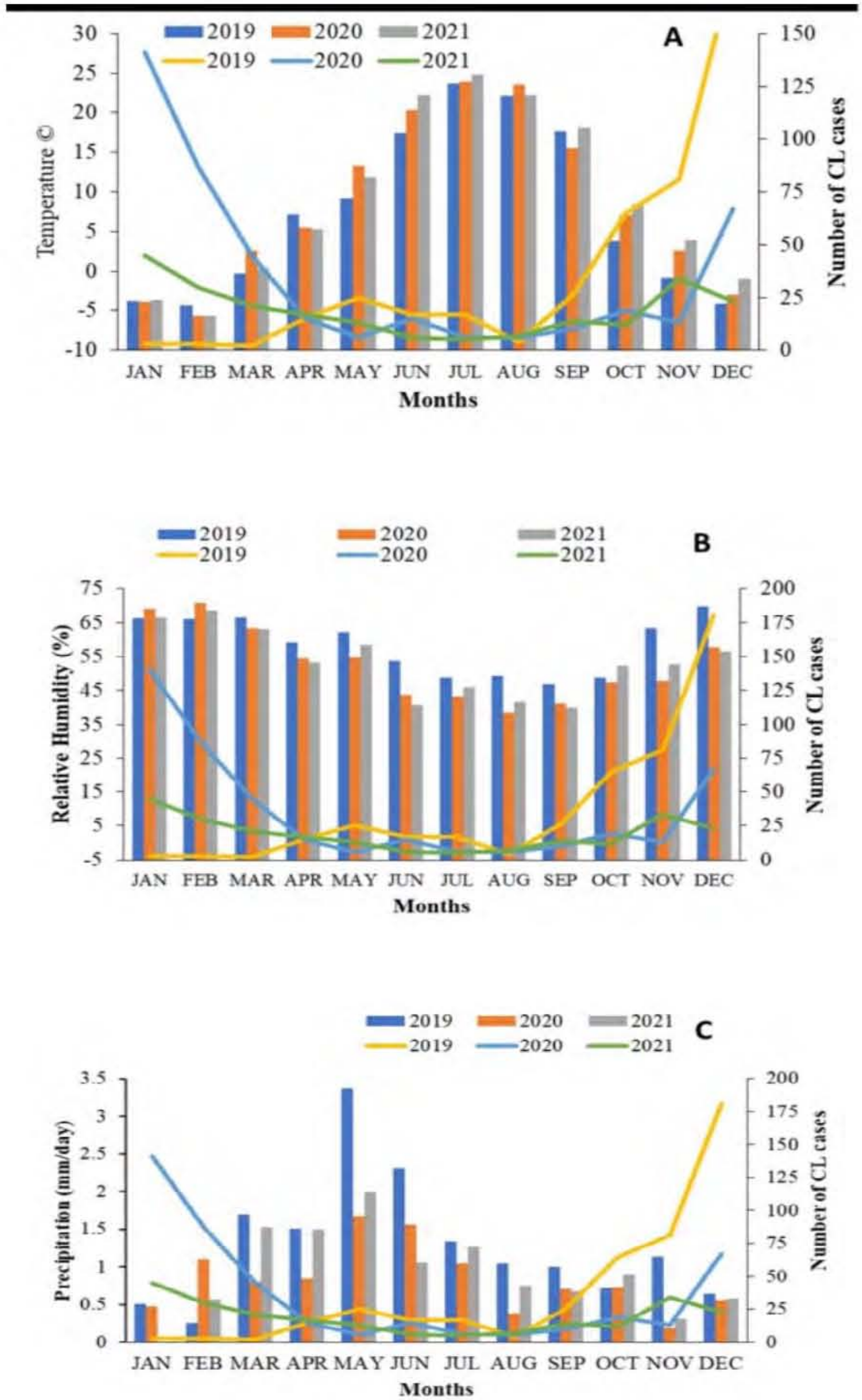

Figure 3.12: Number of CL cases association with mean monthly (A) temperature (°C) (B) relative humidity (%) (C) and precipitation (mm/day) from 2019 to 2021 of D.I.K district of KP, Pakistan.

DISCUSSION

In Pakistan, the dissemination of CL exhibits complex patterns and is constantly spread to surrounding regions. Multiple outbreaks have lately been documented from the northern regions, including Gilgit, Kashmir, and southern KPK. It is believed that the disease-free areas close to these endemic regions' boundaries are also in danger (Rowland et al., 1999); (Zeb et al., 2021).

In our study, CL was discovered to be common in all 5 tehsils of D.I. Khan as well as in the vicinity of contiguous borders. In comparison to the other 4 tehsils, Tehsil D.I.K (62.7%) had the highest percentage of cases, followed by Tehsil Paharpur (9.6%), Paroa (7%), Kulachi (5.8%), and Daraban (1.9%), which had the lowest percentage of cases. CL is widespread in several regions of Pakistan with various meteorological and geographic characteristics. While few instances have also been recorded from Sindh and Punjab, it is largely endemic in KPK (Mujtaba & Khalid, 1998; Sarker et al., 2003). Demographic, socioeconomic, movement from rural to urban areas, religious, cultural, disturbance in climate (flood, drought), environmental factors, and/or insecurity (war/civil convict), people increasingly leaving their hamlets and heading to cities, poor hamlets are major risk factors that represent acute CL (ACL) (Desjeux, 2001). The other major source of risk is cross-border travel. North-western Pakistan experienced an epidemic that started in an Afghan emigrant camp a few years ago. It is thought that infectious migrant carriers of Kabul were the source of outbreak (Rowland et al., 1999).

In present study, most cases originated from the Dera Ismail Khan premises, with localities from various KP regions and a small number of cases coming from Afghan localities currently residing in D.I.K. and its surrounding villages. Another possible explanation is the existence of the DHQ center, which is easily accessible to the residents of D.I.K for the diagnosis and treatment of CL. The majority of people who are sick were between the ages of 1 and 20, based on a recent prevalence of *Leishmania*. Bennis *et al*. (2015) and Haouas *et al*. (2015) observed comparable results, reporting a high rate of infection of roughly 55% among those under the age of 20 as compared to 60 years old, which showed 4% infection in the research of Jamal *et al* (2011). Our results coincide with the majority of those in another research, which revealed the fewest occurrences from those over the age of 61 and the bulk of cases between the ages of 11 and 20

(Bumb et al., 2013; Haouas et al., 2015). As a result, it has been shown that children and individuals with an underdeveloped or weakened immune system are more likely to contract this condition (Bennis et al., 2015; Izadi et al., 2016).

According to current findings, out of a total of 1135 cases, the majority (62%) of cases were involving men, while the percentage of cases involving women was somewhat lower. The number of patients might be the cause of the discrepancy in the findings, while the necessity for expertise when extracting samples from patients could make it feasible for a slide to test negative when employing a microscopic approach. Due to comparable exposure to sandfly bites from infected insects, the current investigation shows an equivalent risk of CL infection in both genders. An epidemiological study by Bettaieb *et al*, (2014) found that CL infection was practically same in both men and women groups. Most studies have revealed that men are more likely to get CL infection Servat *et al*, (2016). Another survey revealed a 24:1 male to female ratio. However, these studies were conducted in Pakistan and India, two nations with comparable societal norms, where only males are mandated to work and have to leave their houses while women must remain at home and only leave the house when deemed needed (Bumb et al., 2013).

In our study there is a range of less than two months to more than a year between the development of the CL lesion and the diagnosis and results are consistent with the study of (Zeb et al., 2021). The patient visited the hospital for the first time on average 12 to 14 weeks after the lesion first developed. All patients (100%) in both groups had early and late lesions, which were recognized as being present for less than six months and more than six months, respectively. Aguado *et al*. (2013) revealed that the majority of CL patients had lesions that last between two and six months in length. The findings are also consistent with those of Abdellatif *et al*. (2013), who found that the majority of lesions were treated within a year.

Our results showed 23.9% people had multiple lesions and 76.1% people had a single lesion, consistent with previous studies (Mubashir Hussain et al., 2017; Shokri et al., 2017). While Ayaz *et al*. (2011) found an increase in the number of lesions with a decrease in the number of cases and noted minimum cases of multiple lesions in fewer patients in comparison to 58% having single lesion and 30% of multiple lesions on each patient, which is quite consistent with our findings, the current research demonstrated that more than half of the patients are infected with only one lesion. Variations in shelter circumstances, food habits, occupational activity, and host immunity to the infection can all lead to changes in the amount of lesions (Mengesha et al., 2014). Similar to current study's finding that the majority of injuries occurred on exposed body regions, including the face and upper and lower body parts was recorded by (Mubashir Hussain et al., 2017). In a study, also observed by Ayaz *et al*, (2011) the majority of the lesions have been primarily observed in face areas. Similar findings were also noted in the majority of studies, and dominating CL lesions on exposed body areas, such as the face, neck, feet, legs, arms, and hands, are caused by exposure to sand fly bites, according to Khan *et al*, (2013).

For topographical estimate of the data obtained from fieldwork or other sources for analysis and prediction of species distributions, mapping, and disease prevalence, computer software programmes like MaxEnt and ArcGIS are frequently used (Enwonwu et al., 2006). Our research suggests that ArcGIS is crucial for creating and converting data for use in software that creates information and evaluates epidemiology (Ostfeld et al., 2005). A number of recent studies have used exceptional resolution three-dimensional satellite photos to map CL and identify possible concern areas at the regional and global levels (Mollalo et al., 2015; Quintana et al., 2013). By precisely assessing the occurrence of CL in the D.I.Khan particularly and overall in KPK Pakistan, current study of (Simon et al., 2011) supported the prior projections with Choropleth map, (Shokri et al., 2017) DEM map, (Moradi-Asl et al., 2017) IDW map, Focal Statistics map by (Pakzad et al., 2017) and mapping Clusters toolset by (Abedi-Astaneh et al., 2016).

During spatial analysis both the temperature and geographic factors were taken into account, a total of 989 CL cases were found dispersed over all of the communities in the district D.I.Khan and adjacent areas from year 2019 to 2022, about 96% of CL cases were discovered in regions with high temperatures, which is in agreement with Kasap *et al* (2009) findings. In addition, temperature has a major role in controlling the morphogenesis and proliferation of disease parasites (Chalghaf et al., 2016; Quiñonez-Díaz et al., 2012). In accordance with the findings of (Guernaoui et al., 2006), whose predictive model suggested that a 5 degree centigrade increase in temperature can dramatically enhance the geographical and aperiodic distribution of sandfly vectors, it is seen that the majority of CL cases were discovered in high temperature regions in this study. This study shows that hotter climates encourage and promote the development of sandfly larvae, which raises the chance of high CL dissemination. In contrast, the emergence of

CL transmission in regions with the lowest temperatures is caused by non-infected residents moving to endemic regions (Artis et al., 2003; Svobodová et al., 2009).

In addition, for vector-borne diseases, the human population is also a component of reproduction number, which has a significant influence in the distribution and transmission of CL. The data indicated that climatic variations significantly influence the dynamics of CL infection (Ready, 2008; Toumi et al., 2012). The seasonality of the disease's incidence pattern suggests that CL transmission is vulnerable to physical circumstances (Chaves & Pascual, 2006). Increased humidity shortens the incubation period, which has a direct impact on the growth and reproduction of the *Leishmania* parasite. Environmental fluctuations and weather either limit or intensify favorable circumstances for disease transmission (Kasap & Alten, 2006).

Conclusion

Leishmaniasis is confirmed to be prevalent in district D.I. Khan by the epidemiological statistics. The prevalence was high among the local population, but a temporal increasing pattern was seen in the tehsil D.I.K. and Paharpur, which suggests a potential danger for the spread of CL. Residents of D.I. Khan and its surrounding areas may face a major danger from CL because the area is very susceptible to the disease due to its dense population. In D.I.Khan KPK, cutaneous Leishmaniasis is becoming more and more prevalent. The findings of this study confirm that mapping is the most accurate method for predicting the prevalence of CL in various geographic areas. However, the forecast accuracy may be improved by incorporating the appropriate environmental and climatic factors. Infection was most common during the winter. The findings indicate that many new incidents of CL are reported in the north-western regions of KPK based on the incidence and distribution of the disease.

Recommendations

- To enable monitoring of CL spatiotemporal patterns and monitor changes in the environment and climate, the implementation of a unified regional monitoring system should be encouraged.
- For disease prevention and management at the individual and community levels, the area needs to get the right attention.

- Another cause is the great distance between hospitals, diagnostic facilities, and remote communities. As a result, there is a need for mobile clinics as well as appropriate education to reduce CL.
- Entomologists are advised to develop measures for controlling the vectors that spread leishmaniasis in light of the current projections.

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