

LEISHMANIA TROPICA A CUTANEOUS DISEASE DETECTED IN PEOPLE RESIDING IN NORTH WAZIRISTAN DISTRIC, KHYBER PAKHTUNKHWA PAKISTAN

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خلاصہ

Cutaneous Leishmaniasis سے مراد ایک ایسا مرض ہے جو ایک مخصوص مکھی (Sandfly) کے کاٹنے اور ایک خلیاتی طفیلے کی منتقلی سے چرے ہاتھوں اور جسم کی جلد پر ظاہر ہوتا ہے۔ 1960 کی دہائی میں اس مرض کو پاکستان میں رپورٹ کیا گیا۔ موجودہ تحقیقی کام خیبر پختون خواہ کے قبائلی اضلاع میں سرانجام دیا گیا جس کا بنیادی مقصد اس مرض کا سبب بننے والے طفیلے کی نشاندہی اور پھیلاؤ کے اسباب جاننا ہے۔ اس تحقیقی کام کے سلسلے میں ضلع شمالی وزیرستان کے مختلف علاقوں سے نمونے اکٹھے کیے گئے۔ اس مقصد کے لیے کل 2873 نمونے اکٹھے کیے گئے اور ان کا تحقیقی مطالعہ کیا گیا۔ تمام نمونوں کی تشخیص کے لیے سلائڈ تیار کیے گئے جس کو جسم کے متاثرہ حصوں سے بائی آپسی کے عمل کے ذریعے سے حاصل کیا گیا تھا۔ خوردبینی مطالعے معلوم ہوا کہ 2873 نمونوں میں سے 1679 (58.44%) اس بیماری سے متاثر پائے گئے جبکہ 1194 (41.55%) میں اس مرض کی تشخیص نہیں ہو سکی۔ تمام مثبت نمونوں میں 973 (55.80%) کی شرح سے مردوں میں یہ مرض پایا گیا جبکہ خواتین میں اس کی شرح 742 (44.19%) فیصد رہی۔ 1 سے 15 سال کی عمر کے لوگ اس مرض سے سب سے زیادہ متاثر پائے گئے۔ جس کی شرح 82.90% بنتی ہے۔ جسم کے متاثرہ حصوں کے مطالعے سے یہ حقائق بھی معلوم ہوئے کہ اس مرض کی وجہ سے زیادہ تر افراد کے چرے متاثر ہوتے ہیں جس کی شرح 59.26% فیصد بنتی ہے۔ اس پورے تحقیقی عمل سے جو حقائق منظر عام پر آئے ان سے معلوم ہوتا ہے کہ ضلع شمالی وزیرستان کے اکثریتی علاقے کو اس بیماری نے شدت سے متاثر کیا ہے۔ ہر مہینے ضلع کے مختلف علاقوں سے اس کے مریض رپورٹ ہوتے رہتے ہیں۔ حکومت اور محکمہ صحت سے استدعا کہ وہ اس سمت میں خصوصی توجہ دیں تاکہ علاقے سے اس خطرناک مرض کا خاتمہ ممکن ہو سکے۔

Abstract

Cutaneous is related to skin disease. The cutaneous leishmaniasis means that a bite of sand fly which produce lesion mainly on face, arm and legs caused by single cell protozoan parasite of order kinetoplastida called leishmania. It was first reported in Pakistan in 1960. The research work was conducted in North Waziristan Tribal District Khyber Pakhtunkhwa Province, Pakistan with main aim to identify and know about the distribution of the parasite. In the current research work samples were collected from different villages of North Waziristan Tribal District. A total of 2873 samples were studied and diagnosed for the presence and absence of LD bodies. All the samples were studied and diagnosed through slide preparation. Smears were prepared by taking the biopsy from the lesion site of the infection. The smear was stained with Giemsa's stain and was studied under light microscope. Out of 2873 samples, the total positive cases were 1679 (58.44%) and negative were 1194 (41.55%). Out of the total 1679 positive samples, 937 were male with 55.80% dominant rate and 742 were female with 44.19% dominant rate. The population of 1-15 years age was most commonly affected by cutaneous leishmaniasis with 82.90% dominancy rate. Based on lesion sites at the body parts analysis shows that face was mainly affected with 59.26%. Single lesions were with high frequency with 89.33% prevalence rate. The whole research work shows that cutaneous leishmaniasis has badly affected the entire North Waziristan Tribal District. Each month numbers of new suspected individuals are reported from different localities of North Waziristan Tribal district. Government and the health concerned authorities are strictly requested to take extra care of the patients of cutaneous leishmaniasis in North Waziristan.

Key Words: Cutaneous; Leishmania; Lesion; North Waziristan.

Introduction

Cutaneous leishmaniasis resulted from the bite of sand fly which produce lesions mainly on the face, arms and legs caused by various type of a single cell protozoan parasite of the order kinetoplastida called leishmania (Butto *et al.*, 2008). There are various species of the genus leishmania which cause leishmaniasis. Each and every species has their own geographical zone (that their characteristics place). These parasites can affect several mammalian species, particularly human. There are more than twenty (20) different species and subspecies of the genus leishmania that can cause leishmaniasis in human. It is one of the zoonotic disease transmitted to the man during a bite of the vector sand fly of the genus *phlebotomies* (Mumtaz *et al.*, 2016).

The infection of leishmaniasis clinically has been divided into the following sub categories; A) cutaneous leishmaniasis (CL); B) diffuse cutaneous leishmaniasis (DCL), C) Mucocutaneous leishmaniasis (MCL); B)

Visceral leishmaniasis (VL). The cutaneous leishmaniasis largely is divided into two (2) forms: an old world cutaneous leishmaniasis which is mainly caused by *leishmania tropica* and *Leishmania major* which are found in central Asia, Middle east, North Africa, North India and Pakistan. While a New world cutaneous leishmaniasis is mainly caused by *leishmania (viannia) braziliensis* and *leishmania Mexicana*, This is widely found in South and Central America along with Brazil (Mebrahtu *et al.*, 1992). The cutaneous leishmaniasis is endemic in over 88 countries and 5 continents of the world. There are estimated 350 million peoples at the risk of leishmaniasis and 5 lac new cases of the visceral leishmaniasis and 15 lac of the Cutaneous leishmaniasis is reported annually (Bhutto *et al.*, 2008).

In America, it is widely spread from Southern Texas to Northern Argentina. Other endemic areas include the Middle East, India, and Pakistan, Iran and North and East Africa (Bari 2006). The cutaneous leishmaniasis in different countries is known by different local names like saladana in Afghanistan, Baghdad sore in Iraq and Kaaldaana in Pakistan it is caused either by *leishmaniatropica* or *leishmania major*. In Pakistan the cutaneous leishmaniasis is an emerging disease (Kassi *et al.*, 2008). The leishmaniasis was first reported in Pakistan in 1960. Initially it was restricted to the northern mountainous region, but now it is widely spreading all over the country (Bhutto *et al.*, 2008).

In Pakistan it is specially found in endemic area near to the adjacent war thorn neighboring Afghanistan and large number of refugees. Beside this it is endemic in Multan, interior Sind and a large area of the Baluchistan (Ahmad *et al.*, 2019). In western regions of Khyber pakhtunkhwa the military operations have given wide range to the cutaneous leishmaniasis in the current years of wars against terrorism (Akram *et al.*, 2015).

Every year 2 million cases of coetaneous leishmaniasis are reported worldwide (Munir *et al.*, 2018). In North West Pakistan cutaneous leishmaniasis is an emerging and serious health problem. The military operations in tribal's areas including current operations in North Waziristan tribal district have given vast space to the incidence and accidents of cutaneous leishmaniasis (Rahim *et al.*, 1998).

On the basis of mode of transmission *cutaneous leishmaniasis* is divided in two types one is Anthroponotic and other one is zoonotic. Anthroponotic (also called urban or dry type) coetaneous leishmaniasis is transmitted from human to human while the zoonotic (also called as rural or wet type) cutaneous leishmaniasis come from human and animal interaction. In Pakistan both zoonotic and anthroponotic cutaneous leishmaniasis is present. In Pakistan the causative agent of the anthroponotic cutaneous leishmaniasis is *leishmaniatropica (L.tropica)* while the zoonotic cutaneous leishmaniasis is caused by *leishmania major (L.major)* (Ahmad *et al.*, 2019). This parasite is transmitted by the biting of phlebotomies sand-fly result nodular lesion is comprised of parasite laden macrophages covered by lymphocytes which try to prevent spread of these organism. This disease is self-healing disease, it takes many months in healing (Butto *et al.*, 2008). The *Leishmaniatropica* and *leishmania major* produce cutaneous ulcers variously known as Oriental sore, Cutaneous leishmaniasis, Jericho boil, and Aleppo boil etc. these two species have similar life cycle but different location, reservoirs and intermediate hosts (Roberts *et al.*, 2013).

Nearly 70 different animals' species are found that are act as reservoirs host for the parasites such as domestic dogs, cattle's, horses, donkeys and rodents. The leishmanial disease may be zoonotic, which include animal reservoir hosts, or anthroponotic where human's reservoir host. Human are considered a prime parasite reservoir in south East Asia and East Africa's (Tiwari *et al.*, 2019). The disease is diagnosed by direct microscopic examination of Giemsa stain smear from the lesion (Khan, 2005). There are about 70 different species of the sand flies which are responsible for the transmission n of the leishmaniasis. Among these 70 species *Lutzomyia* is mainly present in Americas. *Phlebotomies* is present everywhere (Piscopo and Azzopardi 2006).The *leishmania* parasite is transmitted by the biting of the *phlebotomus* sand fly (WHO, 2000). The *phlebotomies* and fly is a vector of the disease and ingest the organism, as an amstigote, come into its digestive tract when sand fly infects the animal. The amastigote develops into promastigote in its digestive tract, and then injected into the susceptible host at the next feed. The promastigote than infects macrophages and develops into amastigotes. The infection is more common in man then woman (Piscopo and Azzopardi, 2006). There are twenty *Leishmania* species that are pathogenic for humans and 30 sand fly species are proven vectors (Bari *et al.*, 2011).

Phlebotomussergenti and *Phlebotomus papatasi* are most common species of sand flies in North Waziristan Tribal District that causes cutaneous leishmaniasis (Mubashir *et al.*, 2018).The lesion manly occurs on the leg, feet, hand, face and neck (Ayaz *et al.*,2018). The cutaneous leishmaniasis usually affects the exposed part of the body because the sand flies easily bite the exposed part of the body and produced ulcer on the biting site (Bari *et al.*, 2011). Present study was aimed to highlight the spread and intensity of cutaneous disease in North Waziristan Tribal District and to emphasize preventive measures by the concern departments.

Materials and Methods

The research work was conducted in North Waziristan Tribal District previously known as North Waziristan Agency. Data was collected from different Tehsils and villages of the study area. Suspected individuals of leishmania disease were studied that were referred to the dermatology outpatient department by the physicians at Central Hospital Miranshah. Then the individuals were diagnosed and tested for the presence and absence of LD bodies in the Medical Laboratory of the Central Hospital Miranshah. All the cases were diagnosed on the basis of clinical findings and smear tests to confirm them whether the case is positive or negative. During the whole work total 3918 cases were studied and were diagnosed through smear tests. The smear tests were performed by taking clean and sterilized fresh slide. Samples were taken from the suspected patients' wound edge and placed on the slide. The samples on the slide were fixed by methanol and then stained with Giemsa's stain. Then the slides were cleaned with tap water, dried and examined under the microscope using 100X oil emersion objective for the presence of LD bodies. The negative cases have been excluded from the result. Data was analyzed by using Microsoft Excel Office Program and the results were expressed in percentages.

Results and Discussion

During present study total 2873 samples were collected from different hospitals of North Waziristan Tribal district. Samples were collected each month from September, 2019 to August, 2020. Out of total 2873 samples 1679 (58.44%) were confirmed positive through diagnosis of the suspected individuals and 1194 (41.55%) samples were negative. Out of the positive 1679 samples 937 (55.80%) were males and 742 (44.19%) were females (Table 1).

Table No.1 Month wise distribution of *Leishmania tropica* in North Waziristan.

Months	No of cases	%age
September	90	5.36
October	117	6.98
November	143	8.51
December	184	10.96
January	207	12.32
February	239	14.27
March	183	10.89
April	118	7.02
May	96	5.71
June	109	6.49
July	113	6.73
August	80	4.76

The sex wise distribution of *Leishmania tropica* in North Waziristan tribal district was also studied. The male 937 (55.80%) were mainly affected as compare to the female 742 (44.19%). The reason behind this fact is that male are mostly outside of the houses for more hours of the day which make them prone to the biting of sand fly. While the females are covered with clothes and shawls which protect them from the biting of sand fly (Table 2).

Table No. 2 Sex wise distribution of *Leishmania tripica* in North Waziristan.

Sexes	Number of cases	%age
Male	937	55.80
Female	742	44.19
Total	1679	100

Site wise distribution of cutaneous leishmaniasis at various parts of the body was identified. Face (59.26%) was the most affected site for biting of sand flies and the dominant percentage ratio indicated that face is the most susceptible site of the body that is mainly prone to the leishmaniasis attack and infection (Table 3).

Table No. 3 Site wise distribution of cutaneous leishmaniasis in North Waziristan

S/No	Site	No of cases	%
1.	Face	995	59.26
2.	Legs	292	17.39
3.	Arm	273	16.25
4.	Nose	08	0.47
5.	Ear	01	0.05
6.	Chest	01	0.05
7.	Multiple site	109	6.49

Similarly, age wise distribution of the parasitic infection was also studied in North Waziristan tribal district. The population of the ages from 1-15 (82.90%) was more affected as compare to other age population. The fact behind this incidence may be that the population of age 1-15 is likely to be careless and unawareness about the facts and background of leishmaniasis (Table 4).

Table No. 4 Age-wise distribution of *Leishmania tropica* infection in North Waziristan

S/No	Age (Years)	Numbers of cases	%age
1.	1-15	1392	82.90
2.	16-25	115	6.84
3.	26-35	70	4.16
4.	36-45	31	1.84
5.	46-80	71	4.22

The frequency and percentage abundance of lesion numbers at the body sites was also determined. It indicates that single lesions with 1350 cases (80.40%) were more dominant as compare to other number of lesions at different parts of the body. The table also indicates that single lesion is followed by double with higher percentage as compare to triple (Table 5).

Table No. 5 Number of lesions at different sites of the body.

S/ no	No of lesion	No of cases	%age
1.	Single	1350	80.40
2.	Double	150	8.93
3.	Triple	119	7.09
4.	Multiple	60	3.58

Nature (wet and scar/dry) and Numbers of lesions were also studied. The results indicates that wet lesions were very dominant as compare to scar lesion. The numbers of wet lesions were 1406 which is 83.7% of the total cases. While dry lesions were 273 (16.2%) of total 1679 cases (Table 6).

Table No. 6. Numbers of wet and dry lesions

S/No	Nature of lesion	Number of cases	%age
1.	Wet	1406	83.7%
2.	Scar/dry	273	16.2%

Cutaneous leishmaniasis (CL), the most widespread form of leishmaniasis, caused by *Leishmania tropica* and *L. major*, has emerged as an endemic disease in Khyber Pakhtunkhwa, Pakistan, (Azizi *et al.*, 2006) owing to frequent movement of internally displaced persons (IDPs) from Waziristan in response to surgical strikes and military operations against terrorists by Pakistani armed forces. Keeping in view the impact of the frequent outbreaks of CL in settled areas, we studied the distribution and identification of *Leishmania tropica* in war-affected North Waziristan, with the help of health department and local government authorities.

It was first reported from Pakistan in 1960 and was limited to northern mountainous region. In Baltistan sand flies were identified that are responsible for the transmission of leishmania species they were; *P. chinensis*, *P. major*, *P. kandelaki* and *P. burneyi*, while here in North Waziristan Tribal District the sand flies which have been identified are *Phlebotomus sergenti* and *Phlebotomus papatasi* (Burney *et al.*, 1979). Moreover, different

species of *Phlebotomus* and *Sergentomya* sand fly genera were identified in both North and South Waziristan; *P. sergenti* was the most abundant species, followed by *P. papatasi*. We reported leishmaniasis infection in 6 female *P. sergenti* and flies. *P. papatasi* is also susceptible to carry *L. tropica* and is widely distributed in different parts of Pakistan; including Khyber Pakhtunkhwa Province (Kilic *et al.*, 1994). The present study was conducted in the selected CL endemic villages of the North Waziristan, Pakistan during 2019-2020. The Waziristan covers 15000 square kilometers with latitude of 33 02 59" N and longitude: 70 01' 10" in Federally Administered Tribal Areas (FATA, 2016).

This disease is present in all four provinces of Pakistan along with Azad Kashmir. Beside this, it is endemic in Multan, Interior Sind and a large part of Balochistan (Afghan *et al.*, 2011). In Pakistan, it is especially endemic in regions adjacent to war thorn neighboring Afghanistan and with large numbers of refugee (Bari *et al.*, 2008).

The research work shows that Khyber Pakhtunkhwa and FATA have been dramatically infected by leishmania species. During the research work dry lesion constitutes 57.6% and wet lesion constitutes 41.2%. While the current study revealed that wet lesion is 16.7% while that dry lesion is 83.7%, the high percentage ratio is probably due to higher rate of secondary infections (Arfan *et al.*, 2011).

A research study was conducted in Sindh province of Pakistan where cutaneous leishmaniasis was endemic in summer season. Same is the case here in North Waziristan Tribal district where cutaneous leishmaniasis is at peak in the months of June, July and August. During research work samples were collected from different localities of North Waziristan Tribal District. The month wise incidence shows that maximum cases have been recorded in the month of July, 2020 (14.03%) and minimum cases have been reported in the month of December, 2019 (3.06%), (Bhutto *et al.*, 2008).

A research work was conducted in Peshawar which concluded that males were (51%) and female were (39.5%). In another study in KPK in Surgul village of Kohat, a similar observation reported that the prevalence was more in male (9.9%) than female (8.3%) (Khan *et al.*, 2016), and the population of age 1-15 years were (43.8%), 16-30 (32.5%), 31-45 (16.7%), and 50+ was (7.0 %) the current research work indicates that males are more infected as compare to females. The prevalence rate in male is 55.80% with 937 cases out of 1679 studied cases and that for female is 44.91% with 752 cases. According to site-wise analysis of collected data indicates that face was the most affected site for lesion. Out of 1679 cases, 59.26% were bite at face by the sand flies. The site-wise incidence that for legs is 17.39%, arm 16.25%, noses 0.47%, ear 0.05%, chest 0.05%, multiple site (6.49%).

The age wise incidence and prevalence of cutaneous leishmaniasis in North Waziristan tribal district showed that the population with 1-15 years ages is more prone to the bite of sand flies. The analysis indicates that the population of age 1-15 is more affected as compare to other aged population. The fact behind this incidence may be that the population of age 1-15 is likely to be careless and unaware about the facts and background of leishmaniasis. The percentage prevalence for different age is; 1-15 years: 1392 cases, (82.90 %), 16-25 years 115 cases (6.84 %), 26-35 years 70 cases (4.16%), 36-45 years 31 cases (1.84 %), and 46-80 years 71 cases (4.22%), (Sami *et al.*, 2009).

According to previous research; single lesion is the most dominant lesion throughout the Waziristan. The current work also indicates that Single lesion is more dominant lesion as compare to other lesions at different parts of the body. Few individuals were double, triple and multiple lesions. The percentage prevalence rate that for lesion is; single lesion 1350 cases (80.40%), double lesion 150 cases (8.93%), triple lesions 119 cases (7.09%), and multiple lesion is 60 cases (3.58%) (Sana *et al.*, 2000).

According to previous study, wet lesions (83.7%) were observed more frequently in Waziristan as compare to dry lesions (16.3%). The current research work also satisfied the fact that wet lesion is more dominant as compare to dry lesion (Nisar, 2002).

Conclusion

It is concluded that males are more vulnerable than that of the females in terms of the *Leishmania tropica* spread in North Waziristan Tribal District. However, 1-15 years age group was commonly infected indicates ignorance to follow health and safety precautions. On the government part, proper screening and preventive measures should be enforced to limit the spread of skin disease.

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