



Research Article

Phlebotominae Sandflies Distribution and Incidence of Leishmaniasis from Selected Areas of Punjab Province, Pakistan

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Authors' Contributions

SN was the chief investigator, collected data, and wrote the manuscript. SI supervised and designed the research work, HA and SS helped in data analysis. SR and MAR critically reviewed and IU removed grammatical and typological mistakes, facilitated in tabulation, submission and corresponding. GP assisted the write-up and technically reviewed it.

Keywords

Sandflies, Risk factors, Leishmania, Prevalence, *Phlebotomus*, *Sergentomyia*

Abstract | Bloodsucking sandflies belonging to dipteran are vectors of human disease called leishmaniasis. It is one of most neglected diseases in our country. The main purpose of the study was to identify the prevalence of sandflies in selected regions of Panjab and their relationship with hospital-reported cases of Leishmaniasis. Clinical information of 124 patients was recorded. Observable factors of prevalence include age group, gender, financial status, clinical symptoms, and travel history. The sandflies also collected and identified. Out of 350 sandflies two genera *Phlebotomus* and *Sergentomyia* were reported, *P. segenti* was most abundant specie and a strong positive linear correlation exists between sandflies collected and the patients reported from the area. It was observed that as the number of sandflies increased, the number of patients with Leishmaniasis increased, and as the number of sand flies decreased, the number of patients decreased. The most affected age group was 1-10 years. The disease was more prevalent in males (57.26) as compared to females (42.74) because males had more travel history and they come into contact with livestock. It is concluded that high rate of prevalence can be related to lack of awareness low socioeconomic level, presence of domestic animals in surroundings, poor hygienic conditions and lack of proper medical care. A public awareness and vaccination program is needed to control the spread factors. There should be an eradicated for breeding sites by keeping the surrounding clean.

Novelty Statement | This study discovered the incidence of Leishmaniasis in selected areas and time period. That can be beneficial for identification and control of sandflies. This study will help the researcher to uncover the critical areas of leishmaniasis and the most common species in Pakistan.

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Introduction

Sandflies Phlebotominae belonging to order Diptera are tiny insects that are of great medical and veterinary

significance. They belong to subfamily Phlebotominae (Gomez, 2005). Which has extensive distribution all over the world present mainly in the tropics and subtropics (Adler, 1957). They are small in size and are blood-sucking dipteran insects. They usually exhibit crepuscular to nocturnal behavior (Lewis, 1982). Adult females of sandflies are blood feeders whereas males do not feed on blood⁴. There are weak fliers and have a basic character

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of hopping flight. Dark and large eyes are present. They have elongated antennae. The mouthparts of sandflies are directed downward in blade-shaped and are very short (Claborn, 2010). Sandflies have dense hairs on their body. When they are at rest, wings are held on back in a special shape which is 'V' shape. The color can range from white to nearly black. The legs of sandflies are longer than body and are very soft. Sandflies are nocturnal in behavior. They are sensitive to dehydration. They accommodate in caves and rocks. They also live in animal burrows, tree holes as well as they can live in human rooms. They are weak fliers and fly near to the ground in short jumps. The flying range of sandflies is 300 m but in some it is observed that in specific environments species fly in range of 2300 m (Lane, 1993). Presently the classification of Phlebotomine sandflies remains debatable. On the basis of innovative classification of Theodor (1948-1958), suggested that there are two genera for Old World species which are *Phlebotomus* and *Sergentomyia* (Young, 1984). Three genera which are proposed for New World species are *Lutzomyia*, *Brumptomyia*, and *Warileya*. Genus *Phlebotomus* consist of 11 subgenera, 96 species and 17 subspecies (Lewis *et al.*, 1977). The name sand fly is misrepresentative because it wrongly recommends to layman that he can be at threat of this vector-borne disease when he visits the beach. In fact, the English meaning represents pale or sandy color of this insect hence giving the name sand fly. A difference should be present for the vectors of the leishmaniasis means sandflies and other flies. So, for distinction term Phlebotomine sandflies are given. Up to 800 species have been projected to be present in diverse regions of the world. They are placed in the suborder Nematocera belonging to the order Diptera. The family is Psychodidae and the subfamily Phlebotominae. Phlebotomine sandflies are mostly distributed in the warm zones of Asia, Africa and Australia. It also includes warm zones of southern Europe and the Americas. The distribution ranges northwards above a latitude of 50 °N in southwest Canada (Young, 1984). It is right below this latitude in northern France and Mongolia⁸. The southernmost distribution of sandflies ends at a latitude of 40°S. Sandflies are absent from New Zealand and the Pacific Islands Lewis (1967). The altitudinal distribution of sandflies ranges from below sea level to 3300 m in Afghanistan (Artemiev, 1980).

The leishmaniasis is a composite of parasitic diseases that cause major health problems on almost all continents of the world. The types of leishmaniasis depend mainly on the species of leishmania which is participating and also depend on the immune response of the human host (Thomas *et al.*, 1992). The most populated province of Pakistan is Punjab having about 50% population of country (Kendrick, 1999). It is endemic for leishmaniasis (Bari, 2012). About 29 species of sandflies that act as vector of disease are present in Pakistan (Young, 1984). The present study investigated phlebotomine sandflies distribution and

incidence of Leishmaniasis from selected areas of Punjab Province, Pakistan.

Materials and Methods

The study was conducted on sandflies collected from Rawalpindi and other selected areas of Punjab province, Pakistan. The leishmaniasis patient's data was collected from hospitals and labs. The patients of leishmaniasis that were interviewed came from different areas of Rawalpindi such as from Fateh Jang, Taxila, Gujar Khan, Kotli Sattian and Murree. The study was conducted for nine months from September, 2018 to May, 2019 in Rawalpindi.

The sandflies were collected and the information was taken from patients of leishmaniasis by interviewing them. The data of patients were collected from the hospitals. Patients were included in the survey so that awareness level can also be checked. Different environmental factors were also observed. The age range of patients was 1-70 years. The collected specimens of sandflies were preserved and identified with the help of available literature and identification keys.

Collection of sand flies was carried out during nine months by using sticky traps that were prepared by using A-4 sized white papers smeared with castor oil. On average 15 traps were hanged per day at a height of 30cm above the ground. The traps were hanged with the help of thread in different habitats such as in herds of cows, goats and sheep's, bird's cages, indoor places, in the crevices of walls and in the dense green vegetation before the sunset and were recovered after the sunrise. The collection of sandflies was done from the cattle dungs, cracks in the walls, and houses of people having domestic animals. The collection sites were both indoor and outdoor.

Preservation

The sandflies were recovered from the traps. With the help of very fine needles, they were picked and then dipped in 70% ethanol. After dipping in ethanol, oil on the bodies of sandflies was removed. For that purpose, they were washed for 2-5 minutes. The collected specimens were stored in plastic tubes in 70% ethanol (Emami and Yazdi, 2008). The tubes were marked and labeled on the day of collection. The information regarding specimen was mentioned on it. The date, time and site of collection was also mentioned. In this way record was maintained. The total number of flies collected per month was mentioned on separate jar.

Identification

Sandflies were recognized and identified on the basis of external morphology such as size 1.5-2 mm, absence of an eye bridge between eyes, cylindrical flagellomeres on antenna which are about 14 in number, 5 palpomeres of

palps, mouthparts such as proboscis equal to the length of head, legs longer than body, color of cuticle is light brown to black, almost erect wings and wings venation is five branched radial vein on wings. Specimens were recognized as males or females on the basis of morphology of abdomen. In case of females, the last tergite of abdomen looks blunt but in case of external genitalia on the tip of abdomen. The next step was to separate the sandflies on the basis of genus. Specimens of genus *Sergentomyia* were separated from *Phlebotomus* on the basis of small body size, presence of recumbent hairs on abdomen, tear-shaped sockets on tergites 2-6, narrow and lanceolate wings, presence of definite row of teeth in ciborium, presence of pigment patch and presence of all terminal spines in case of males.

Members of genus *Phlebotomus* possessed comparatively large body size, erect hairs on abdomen, spherical sockets, broad and asymmetrical wings, absence of teeth in the ciborium, absence of pigment patch and some basal spines along with terminal spines in case of males. Identification was carried out by consulting the published literature by Jumms khan-kakkarsulemankhel. Standard keys for identification of sand fly were consulted along with other available literature (Artemiev, 1980; Lewis, 1982).

Patients data

A survey was carried out in the different areas of Rawalpindi, Punjab. Patients of leishmaniasis were included in this study. The data was collected from different hospitals and labs Rawalpindi and other selected areas. The data of patients and relevant clinical information was recorded. Environmental conditions were checked so that relevance with disease can find out. The prevalence of leishmaniasis was examined in relation to age group, gender, clinical symptoms and traveling of patients in present and past. The information related to economic status, home type, site of lesions, presence of domestic animals in surroundings was also taken.

Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2010 XP and SPSS software. The statistic was applied to find out the distribution of sandflies and the prevalence of leishmaniasis in the study area. Pearson's correlation was applied to the data to find out the association between monthly collection of sandflies in the area and the number of patients reported per month. Values were considered to be statistically significant when the p-value was less than 0.05.

Results

Collection of patients data

There was a greater percentage of males infected

(58%) compared to females (42%). This is because of more exposure of males to surroundings as compared to females. Also, males had more travel history exposure to domestic animals. Females mostly cover their bodies by wearing full sleeves and stay mostly inside houses so they were less infected in areas being observed. The most affected age group was children of 1-10 years including more percentage of males as compared to females. The least infected age group was 61-70. Most of the patients were suffering from multiple lesions as compared to single lesions. The majority of patients were having lesions on hands, face, legs, arms and other body parts, Males had more exposure to sandflies bites so they had more chances to suffer from disease. About 34.68% patients were having lesions on hands and arms. The hotspot of lesions was hands and arms of patients. Other body parts were observed with fewer lesions. Out of 124 patients, 36 patients had lesions on their feet and legs.

Collection of sandflies

The highest collection was observed in month of December which was followed by November. The lowest number of sandflies was collected in month of February. There is increase in number of sandflies from September to December. After that there is decrease in sandflies number in January. The least number of sandflies were collected in the month of February as shown in Table 1.

Table 1: Monthly collection of sandflies.

Sr. No.	Month	Year	Number of sandflies
1	September	2018	38
2	October	2018	42
3	November	2018	47
4	December	2018	52
5	January	2018	44
6	February	2019	23
7	March	2019	28
8	April	2019	34
9	May	2019	42
	Total		350

Species of sandflies

A total of 350 sandflies was collected from the study area belonging to two genera, *Phlebotomus* and *Sergentomyia*. The most abundant species from the collection was *P. sergenti*. Standard keys for identification of sandflies. The species of sandflies which were collected and identified during the study period were *P. papatasi*, *P. sergenti*, *P. major*, *P. nuri*, *P. keshbishiani*, *S. Baghdadis*, *S. babu*, *S. hospitti*, *S. heodori*, *S. clydei*, and *S. montana*. *P. sergenti* was most abundant specie as shown in Figure 1.

Correlation between sandflies and patients

The increase in the number of sandflies, there was

increase in patients of leishmaniasis and with decrease in number of sandflies there was decrease in number of patients. So, to control the disease, breeding sites of sandflies should be eradicated. The value of Pearson's Correlation was 0.554 and level of significance was 0.021 represented by Figure 2. The value is statistically significant which means that there exists a strong positive relationship.

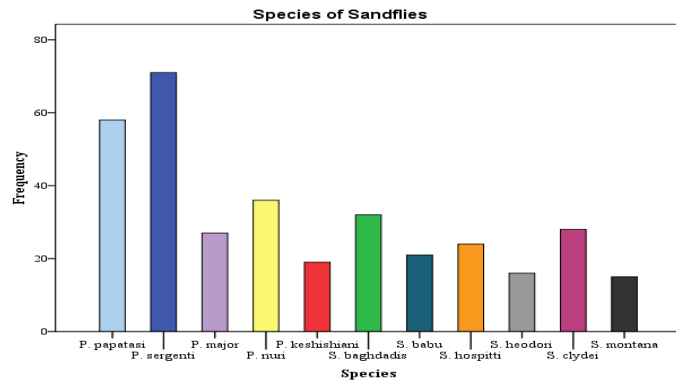


Figure 1: Species of sandflies.

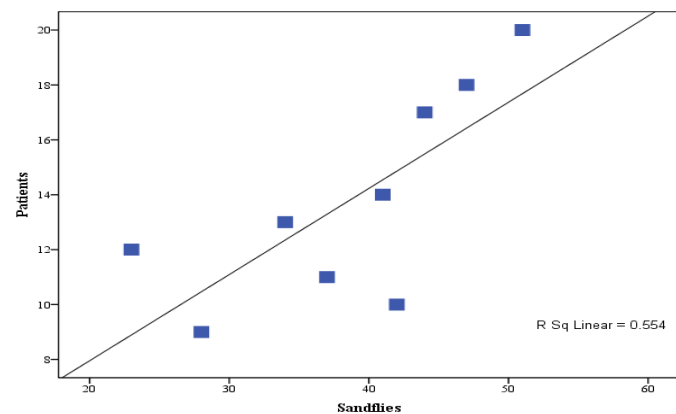


Figure 2: Correlation between sandflies and patients.

Discussion

It was observed that people belonging to the lower class have more risk of leishmaniasis as compared to middle class and upper class. The sanitary conditions of lower-class have greater risk of disease. Poverty enhances the rate of leishmaniasis by increasing the risk factors of the disease. Due to poor sanitary conditions the number of sandflies increases because the breeding sites increases and ultimately the access of sandflies to human also increase. As lower-class was more affected by this disease, so the case was observed with type of houses in which they live. The people were affected due to poor sanitary conditions (Mughal, 2014; Shaheen *et al.*, 2020). Most of them had temporary structures of the house such as made up of mud or simply a tent was used. Most of the patients had domestic animals in their surrounding or they had kept them in their houses. They had mostly cows and dogs in their surroundings. Males had more exposure to domestic animals so they were suffering from leishmaniasis. Few

patients had not petted in their houses or they had no exposure to animals. The same results were observed in a study Shakila *et al.*, 2006.

The types of sandflies collected and identified during the study were: *P. papatasi*, *P. sergenti*, *P. major*, *P. nuri*, *P. keshishiani*, *S. Baghdadis*, *S. babu*, *S. hospitti*, *S. heodori*, *S. clydei*, and *S. montana*. The blue bar graph in Figure 2 shows that *P. sergenti* has the most species. These species have also been previously reported during the geographical distribution of sand flies (Khalid *et al.*, 2012). Men (57.26%) were infected with Leishmaniasis more than women (42.74%). This is due to greater exposure to the male environment. Males have more travel history and they have been exposed to domestic animals. Men are more affected than women (Khalid *et al.*, 2012; Al-Aubaidi *et al.*, 2016). The present result showed that the most affected age group is children aged 1-10 years. It has been observed that men and children are mostly infected. In the prevalence study of Leishmaniasis, children are most affected by the disease (Mughal, 2014).

To avoid the disease proper clothes should be worn so cover full body parts, less exposure to surrounding especially at night because sandflies are active at night. Proper nets should be used and insecticides and repellants should also be used. Proper vaccination is much needed for leishmaniasis. It is observed that people are unaware of this disease. Seminars and campaigns should be organized so that people have knowledge about this disease. Our media should realize the importance of this neglected disease and can aware the people to protect themselves. Different control methods should be used to avoid the risk of disease. Breeding sites of sandflies should be eradicated such as by keeping the surrounding clean and removal of garbage and organic matter. Waste should be properly disposed. The cracks of doors and windows should be covered properly. Keep the environment clean. It was concluded that with the increase in number of sandflies, there was increase in patients of leishmaniasis and with decrease in number of sandflies there was decrease in number of patients. So, to control the disease, breeding sites of sandflies should be eradicated. The value of Pearson's Correlation was 0.554 and level of significance was 0.021. The value is statistically significant which means that there exists a strong positive relationship.

Conclusion

It was concluded that there exists a strong correlation between sandflies collected and the patients reported from the study area. With the increase in the number of sandflies, there was increase in patients of leishmaniasis. The high rate of prevalence is due to poor economic level, presence of domestic animals in surroundings, personal hygienic conditions, less awareness of the disease and

lack of proper treatment to disease. The changes made by man on the earth for his interest and needs has resulted in more exposure of man to the sandflies. Deforestation and sanitary conditions have also enhanced the risk of disease. Due to poor sanitary conditions the number of sandflies increases because the breeding sites increases and ultimately the access of sandflies to human also increase. The value of Pearson's Correlation was .745 and level of significance was .021. The value is statistically significant which means that there exists a strong positive relationship.

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Conflict of interest

The authors have declared no conflict of interest.

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