

PREVALENCE AND ANALYSIS OF CUTANEOUS LEISHMANIASIS: A COMPARATIVE STUDY BETWEEN GENDERS IN DIFFERENT AGE GROUPS IN THE TRIPOLI AREA, LIBYA.

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Abstract

Leishmaniasis comprises a crucial complex of protozoa vector-borne diseases affecting both humans and animals. It can be caused by various species of *Leishmania*. While a few of these organisms primarily thrive in humans, the majority circulate predominantly in animals, with most being zoonotic. Transmission of Leishmaniasis occurs through sandflies, making prevention challenging. Additionally, some drugs used for treatment pose significant side effects or have limited availability outside endemic regions. Cutaneous leishmaniasis manifests as ulcers, macules, papules, or nodules on the skin caused by *Leishmania*. The recent increase in cutaneous leishmaniasis infections is attributed to apparent lethargy, exacerbated by the instability and chaos in the country. This upward trend is a perilous indicator, underscoring the urgency of conducting extensive studies on this endemic disease. **The aim** of the present study is identify infection rates among males and females of various age groups in the study area and ascertain the extent of the disease's prevalence in the Libyan West and determine its annual increase in the study areas. **Material and methods** for the cases were distributed across 12 regions during three time periods (2020, 2021, & 2022). Sample data were collected from individuals at the National Center for Communicable Diseases Control in Tripoli, **the results** shown that the number of cases for Cutaneous Leishmaniasis was 497. These cases were classified into males and females, with 310 cases recorded for males and 187 for females, according to data it were 62.37% for males and 37.63% for females. **In conclusion** the age group 41-50 exhibited the highest rate of infection, it can be concluded that several key features require consideration. Lastly, but importantly, there is a need to raise awareness among the population about the severity of this disease and disseminate health advice to prevent infection with this epidemic.

Keywords: Cutaneous Leishmaniasis; transmission; infections; protozoa parasites.

1. INTRODUCTION

Cutaneous Leishmaniasis is an endemic disease in the tropics and neo-tropics. It is often classified as a group of diseases due to the varied spectrum of clinical manifestations, ranging from small cutaneous nodules to extensive mucosal tissue destruction. Cutaneous Leishmaniasis can be caused by several *Leishmania* species and is transmitted to both humans and animals by sandflies (Alemayehu, 2017; Oryan & Akbari, 2016; Magill 2010). The diverse clinical presentations, encompassing small cutaneous nodules to severe mucosal tissue destruction, contribute to its characterization as a group of diseases. Cutaneous Leishmaniasis is endemic in both the Old World and the New World. In the Old World, such as Spain and the Mediterranean and Caspian Sea areas, the main reservoir of Cutaneous Leishmaniasis is the dog. Cutaneous Leishmaniasis in the Old World is caused by *L. major*, *L. tropical*, and *L. aethiopica* whereas in the New World, members of the *L. mexicana* complex, *L. braziliensis*, *L. peruviana*, *L. panamensis* in the *Viannia* subgenus, as well as *L. chagasi* and *L. major* (Pratlong et al., 2009; Albert et al., 2009). Despite its increasing worldwide incidence, Cutaneous Leishmaniasis being rarely fatal, has become one of the so-called neglected diseases. There is little interest from financial donors, public-health authorities, and professionals to implement activities for researching, preventing, or controlling the disease. In endemic countries, diagnosis is often made clinically and, if possible, by microscopic examination of lesion biopsy smears to visually confirm *Leishmania* parasites as the cause. The use of more sophisticated diagnostic techniques that allow for species identification is usually restricted to research or clinical settings in non-endemic countries (Sigrid et al., 2005; Convit et al 1993).

The mainstays of Cutaneous Leishmaniasis treatment are pentavalent antimonials, with new oral and topical treatment alternatives only becoming available in the past few years; a vaccine currently does not exist. Disease prevention and control are challenging due to the complexity of Cutaneous Leishmaniasis epidemiology and the limited options available for effective vector control (Reithinger et al., 2007). The incidence of Cutaneous Leishmaniasis has recently surged, likely due to the apparent lethargy stemming from the instability and chaos in the country. This trend is perilous, and if it persists, it poses a significant threat. Therefore, there is an imperative need to conduct comprehensive studies on this endemic disease.

The objective of the study

To ascertain the extent of the disease's prevalence in the Libyan West and determine its annual increase in the study areas. Additionally, the study aims to identify infection rates among males and females of various age groups in the study area.

Material and Methods

Place and period of Study

These cases were distributed across 12 regions during three time periods (2020, 2021, & 2022) and consisted of 62.37% males and 37.63% females. Sample data were collected from individuals at the National Center for Communicable Diseases Control in Tripoli.

Study population

The study population comprised 497 cases of cutaneous Cutaneous Leishmaniasis and data were collected from the National Center for Communicable Diseases Control in Tripoli, Libya. Several methods are available for diagnosing Cutaneous Leishmaniasis, including biopsy, punch or scraping of lesion fragments, and serum for antibody detection. Before obtaining specimens, the skin is prepared by injecting anesthetic (e.g., 1% lidocaine with epinephrine 1:100,000) through intact skin that has been cleansed with 70% alcohol into the dermis underlying the area to be sampled. It is essential to avoid high concentrations of anesthetic, which could inhibit parasite growth in culture.

- Thoroughly cleanse the relevant area of the skin (e.g., with 70% alcohol). It is preferable not to use iodine, as it could inhibit parasite growth in culture. If iodine is used, it should be thoroughly washed off.
- If biopsy specimens and/or dermal scrapings will be obtained, use a scalpel blade to debride scabs and devitalized tissue from the relevant areas. Then, apply pressure with sterile gauze to achieve hemostasis.

Types of Specimens

Following are some types of specimens:

- i. Biopsy specimens.
- ii. Impression smears.

- iii. Needle aspirates.
- iv. Dermal scrapings.

Types of diagnosing test

Following are types of diagnosing test:

Microscopic Examination

Microscopic examination is conducted by collecting a sample from a patient with Cutaneous Leishmaniasis. First, the lesion is cleaned with 70% alcohol. Secondly, the skin is scraped with a medical scalpel, and the scraping is done in one direction until the blood is removed. A smear is then collected from the scraped skin tissue of the patient. The blot is prepared by staining it with Giemsa or Hematoxylin and Eosin (H&E) and viewed under a microscope (100x) for the presence of amastigotes.

DNA Isolation

Biopsy tissue is collected and placed directly in a clear solution (150 mmol/L NaCl, 15 mmol/L Tris-HCl pH 8.3, and 1 mmol/L EDTA) to isolate DNA. DNA is then isolated from parasite cultures and lesion biopsy swabs through overnight lysis in NET buffer containing proteinase K (Sigma) and 1% sodium dodecyl sulfate.

PCR Amplification

PCR amplification was performed using genus-specific primers targeting kinetoplast DN (kDNA). The primer set, consisting of a forward first-step primer and a second-step reverse primer, was utilized for a nested PCR carried out in two amplification steps within the same tube. The PCR mix comprised 0.2 ml Taq DNA polymerase (5 units/ml), 5 ml of 10 PCR buffer, 1.25 ml dimethyl sulfoxide (DMSO), 4 ml of 10 mM deoxyribonucleotide triphosphates (dNTPs; 2.5 mM each), and 2.5 ml of each primer (10 pmol/ml), resulting in a final volume of 50 ml. For PCR amplification, 1 ml of reference strain DNA (85–90 ng) or 4 ml of culture or smear sample was used as the DNA template. The cycling conditions were 94 °C for 30 s, followed by 30 cycles of 94 °C for 30 s, 55 °C for 60 s, and a final extension cycle of 72 °C for 90 s. One microliter of a 9:1 dilution of the first-round product in distilled H₂O served as the template for the second round in a total volume of 50 ml, under the same conditions as those for the first round, except with the second-step reverse primer.

Results

Data was collected for the years 2020, 2021, and 2022 from the National Center for Disease Control in Tripoli. The number of cases for Cutaneous Leishmaniasis was 497. These cases were classified into males and females, with 310 cases recorded for males and 187 for females, as shown in Figure 1.

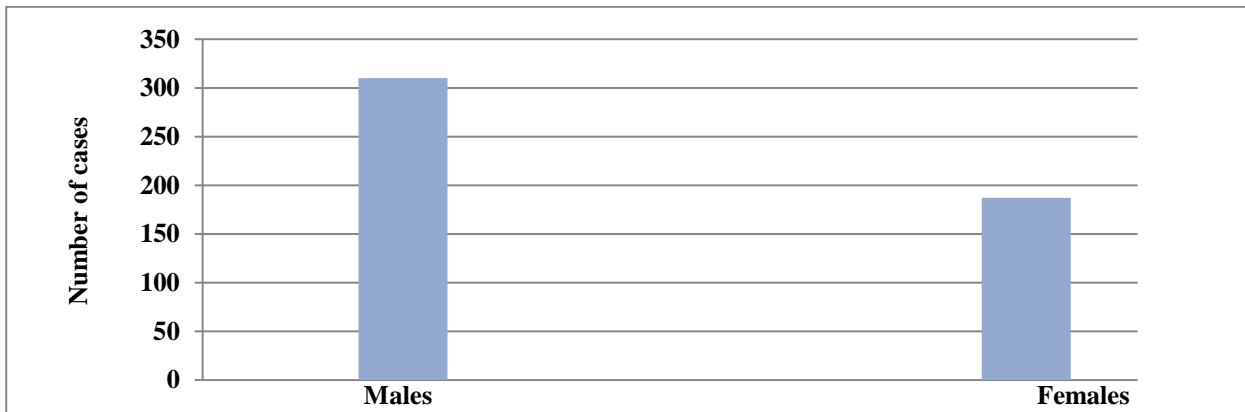


Fig. 1: The Total Number of Males and Females

According to data it is 62.37% for males and 37.63% for females, as shown in the Figure 2.

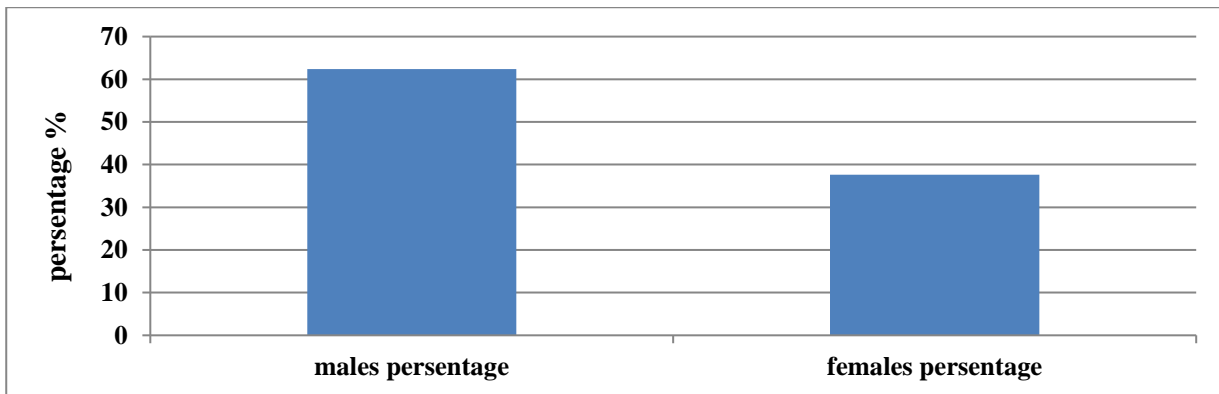


Fig. 2: The Ratio of Males and Females

In the year 2020, the total number of cases was 349, marking the highest infection rate compared to other years. In 2021, the total number of referrals was 104 cases, and in 2022, the count was lower, with 44 cases. This decrease can be attributed to the fact that samples were taken in the middle of the first year, as illustrated in Figure 3.

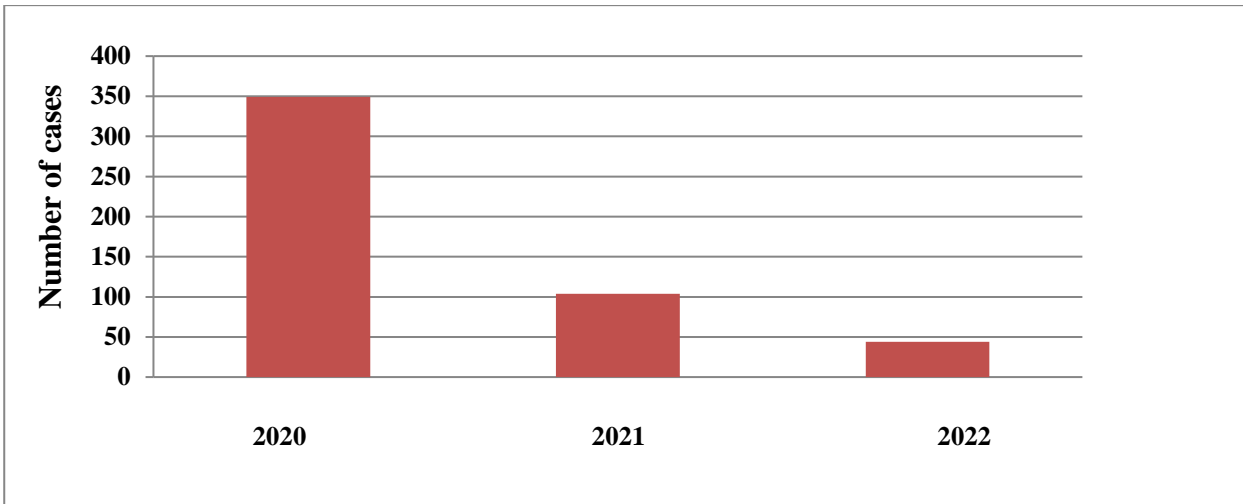


Fig. 3: Total Number of Cases during Three Years

These referrals were distributed throughout the months of the year 2020. It was observed that the incidence of CL showed an increase in the first three months January, February, and March compared to the months from May to October. This pattern is illustrated in Figure 4.

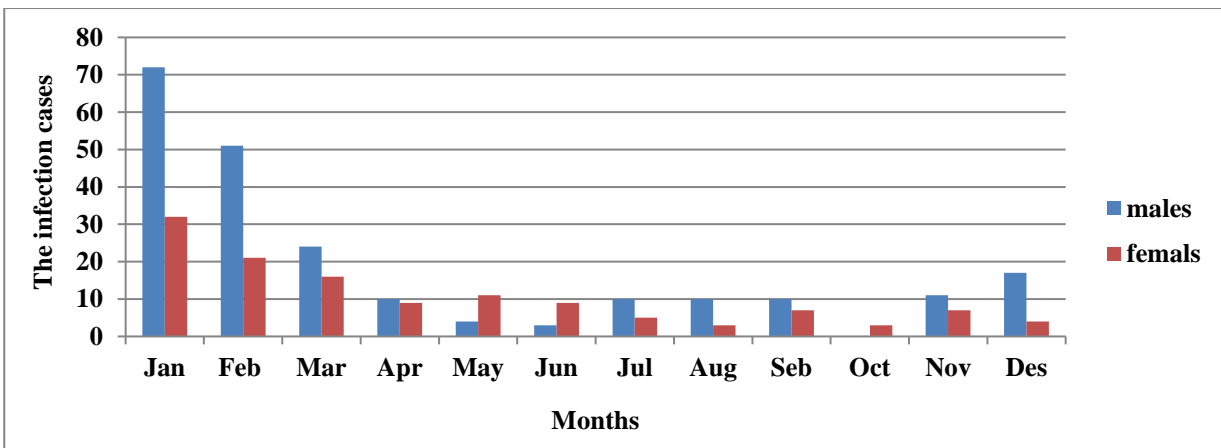


Fig. 4: Cases of Infection during the Months of the Year 2020

In 2021, the infection rate was lower than in 2020. The highest infection rate for this year was also recorded in the initial months, with no cases reported in January, February and July. The lowest infection rate occurred during the months from May to November, as depicted in Figure 5.

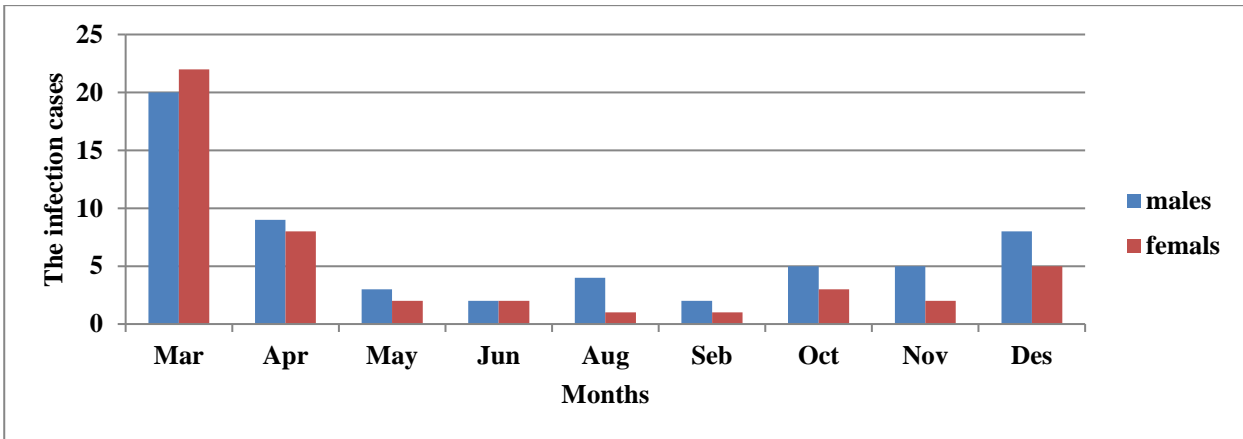


Fig. 5: Cases of Infection during the Months of the Year 2021

Whereas, in the year 2022, the number of cases is considered to be lower due to the instances recorded in the first half of the year. Similar to other cases documented, there is an average in the initial months, as illustrated in Figure 6.

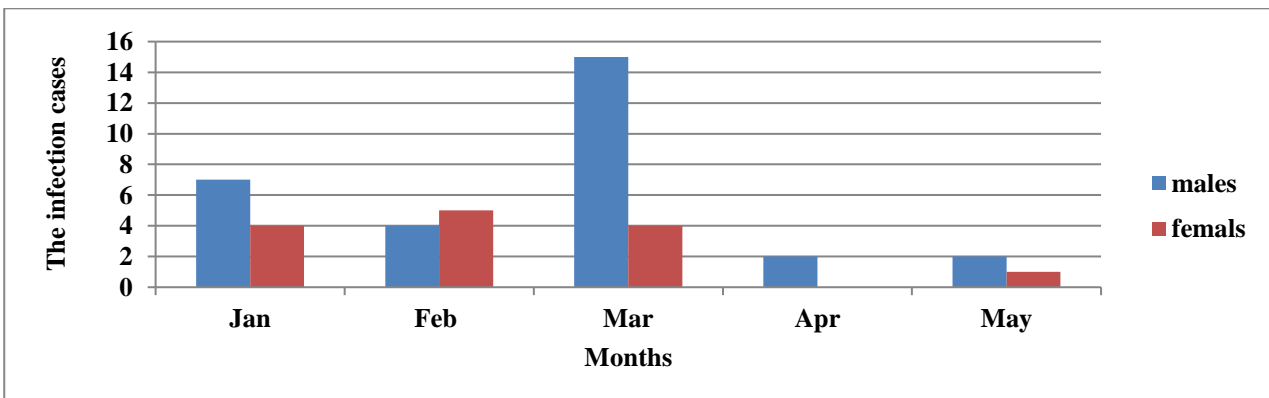


Fig. 6: Cases of Infection during the Months of the Year 2022

The cases were also categorized into age groups ranging from 0-10 years to 81-90 years to determine which age groups are more affected than others. It was observed that the highest percentage occurred in the age group 41-50 in all years, while the lowest percentage was in the older age groups from 70 to 90, as depicted in Figures 7(a), 7(b), and 7(c).

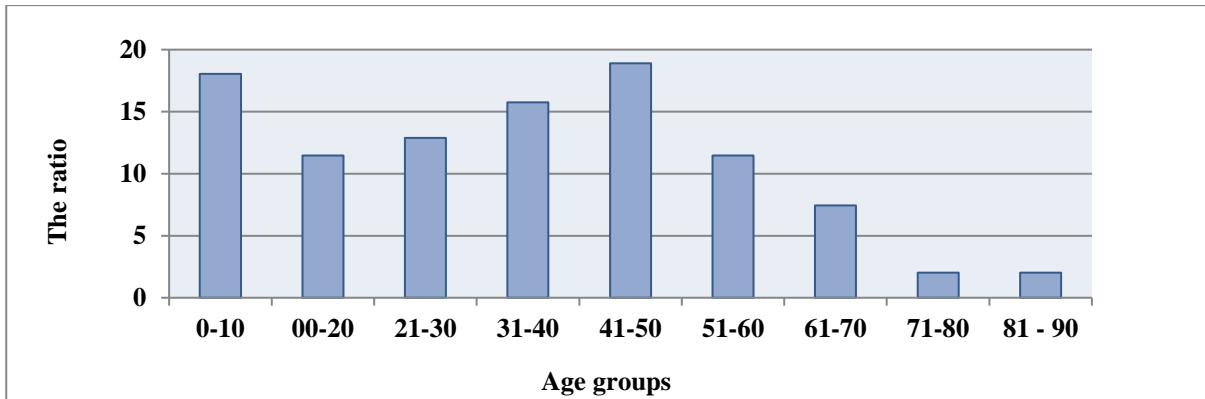


Fig. 7 (a): shows the percentages of different age groups in 2020

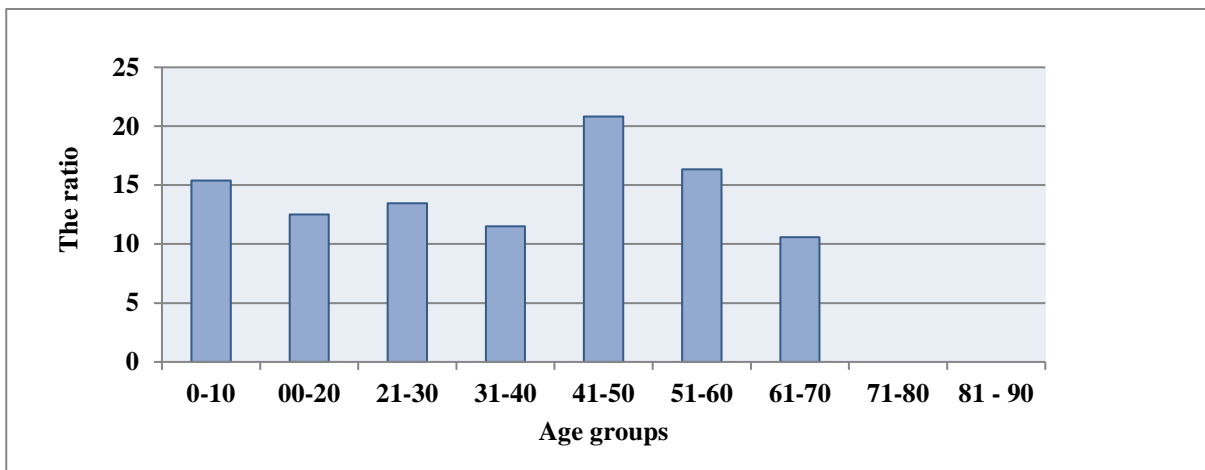


Fig. 7 (b): Percentages of Different Age Groups in 2021

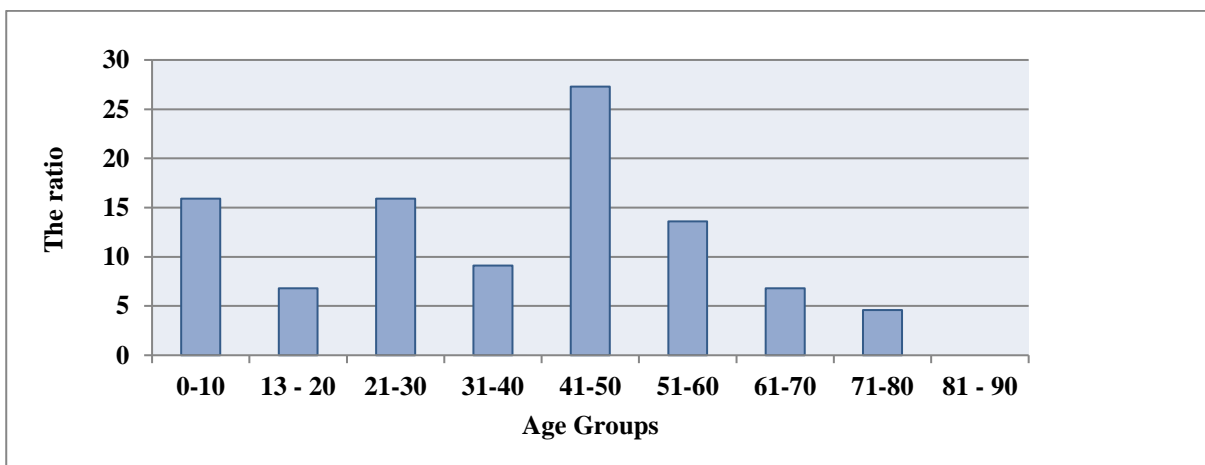


Fig. 7 (c): Percentages of Different Age Groups in 2022

Discussion

Cutaneous Leishmaniasis is a disease that has spread globally, impacting numerous people, particularly in the western region of Libya. Given its significant impact, numerous studies and research endeavors have been conducted. The findings from the National Center for Disease Control in Tripoli during the years 2020, 2021, and 2022 indicate that the number of infections in males is higher than in females. This trend is attributed to societal customs in our country, a pattern also observed in other studies conducted by Manal et al. (2013).

The highest number of infection cases occurred in the year 2020, surpassing the figures for 2021 and 2022. This could be attributed to the war that erupted in 2019, prompting many residents of Tripoli to return to their hometowns in the mountainous and countryside areas where this epidemic is prevalent. In 2021, there was a notable increase in infections compared to 2022, which is not yet complete. Additionally, it was observed that the first three months of each year consistently exhibited higher infection rates, despite the inactivity of sand flies during the cold winter months. Symptoms tend to appear at the onset of winter, aligning with findings from various studies in this field. The cases of the disease were categorized into age groups for comparative analysis. The studied data reveals that the age group 41-50 experienced the highest infection rate, a pattern also observed in other studies conducted by Tarek et al (2017). seasonal activity of Cutaneous Leishmaniasis infection was observed that showed a clear peak between November and January, this is fully consistent with the study conducted by Amro et al(2012). It's important to note that this doesn't imply other age groups are immune; rather, it indicates that the majority of cases, based on the collected data, occurred in the age range of 10-50 as highlighted in the results.

Conclusion

It was observed from the obtained data that the annual increase is not clearly discernible, mainly due to the challenges in obtaining regular and successive data. However, some of the acquired data provides valuable insights, allowing us to address specific research objectives. Notably, the year 2020 reported the highest number of infection cases compared to 2021 and 2022. Moreover, there is a discernible trend indicating that the number of infections is higher in males than females. Additionally, the age group 41-50 exhibited the highest rate of infection. In light of the above discussion, it can be concluded that several key features require consideration. Firstly, there is a necessity for conducting more studies on Cutaneous Leishmaniasis in endemic areas. Secondly, controlling the carrier host, which is the sand

flies, can be achieved through methods such as spraying or eliminating their breeding sites. Thirdly, combating the intermediate host can involve actions like plowing the land to disrupt their habitats. Lastly, but importantly, there is a need to raise awareness among the population about the severity of this disease and disseminate health advice to prevent infection with this epidemic.

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