

<http://doi.org/10.32792/utq.jceps.10.01.01>

The effect of diabetes on the development of skin lesions in patients in infected with cutaneous leishmaniasis in Thi-Qar province , Iraq.

Zainab K. Aouda ¹, Zainab A. Mohammad ², Hind M. Mousa ³

zainab-kha@utq.edu.iq¹ zainababdali6@gmail.com² Hindmousa_pa@sci.utq.edu.iq

^{1,2} Biology Department ,College of Education for Pure Science, University of Thi-Qar, Nasiriyah / Iraq

³Pathological Analysis Department ,Faculty of Science, University of Thi-Qar, Nasiriyah / Iraq

Received 13/12/2023 Accepted 30/1/ 2024, Published 1/3/2024



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

Abstract:

Cutaneous Leishmaniasis is the most common form of Leishmaniasis, it affects the skin and cause a painless and chronic papule at the site of the infected sand fly bite. The current study aimed to assess the associated-risk determinants for cutaneous leishmaniasis in patients with diabetes compared to patients without diabetes. The direct stain method used to diagnose the cutaneous leishmaniasis. Blood samples were collected from 45 confirmed cutaneous leishmaniasis patients for the purpose of measuring HBA1C for patients with cutaneous leishmaniasis. The results of current study showed that 276 out of 315 (87.61%) were infected with *cutaneous leishmaniasis* by microscopic examination. Significant differences ($P < 0.05$) were recorded in the prevalence of cutaneous leishmaniasis according to patient sex and the infected males 65.21% more than infected females 34.78%. Eleven out of 45 cutaneous leishmaniasis patients were suffered from diabetes with prevalence 24.44%. A high association between diabetes and increase in the size of the skin lesions was recorded in current study, the prevalence of diabetic patients with large skin lesions 17.78% higher than the prevalence of diabetic patients with small skin lesions 6.67%, also an association between diabetes and increased the number of cutaneous skin lesions was reported. The prevalence of cutaneous leishmaniasis patients with diabetes who suffered from multiple skin lesions was 13.33% higher than the prevalence of cutaneous leishmaniasis patients with diabetes who had a single skin lesions 11.11% and an association between diabetes and non-response to treatment of cutaneous leishmaniasis patients was recorded, the prevalence of cutaneous leishmaniasis patients with diabetes who did not respond to treatment was 15.56% higher than the prevalence of cutaneous leishmaniasis patients with diabetes who responded to treatment 8.89%. The results of current study demonstrated a significant relationship between diabetes and cutaneous leishmaniasis in distinct risk determinants. Also, the study showed that the diabetes increased the severity of active cutaneous leishmaniasis.

Keywords: : leishmaniasis, cutaneous leishmaniasis, diabetes, Iraq.

Introduction:

Cutaneous Leishmaniasis is the most common form of Leishmaniasis, it affects the skin and cause a painless and chronic papule at the site of the infected female sand fly bite [1]. *Leishmania major* and *Leishmania tropica* are the causative agents of cutaneous leishmaniasis in Iraq, the less severe form caused self healing lesions[2]. The disease is transmitted by a female sand fly infected with *Leishmania* parasites, the infection begins in the form of papules that increase in size to produce nodules, ulcers, and crusts with different symptoms according to the type of parasite and the immune response of patients and the geograpgical regions [3]. After an incubation period (several months) a skin lesion appears, which often heals spontaneously to leave a permanent scar and immunity [4]. Cutaneous *Leishmania* parasite lives inside the macrophage cells of the vertebrate host in the form of amastigotes, and in the intestines of the sand fly *Phlebotomus* spp. in the from of the promastigote[5]. About 30 species of sand flies become infected when they take their meal from the blood of a host infected with the parasites, such as humans, or reservoir hosts, such as wild animals such as rodents and domestic animals such as dogs, goats, and cats [6]. The diagnosis of cutaneous leishmaniasis depends on the appearance of the parasite in a swab or skin biopsy sample through direct microscopic examination and immunological examination. In recent years, PCR technology has been successfully used to detect *Leishmania* species [7]. The current study amied to assess the associated-risk determinants for cutaneous leishmaniasis in patients with diabetes compared to patients without diabetes.

Materials and methods:

-Collection of Samples:

About 315 samples were collected from patients who clinically suspected with cutaneous leishmaniasis in the Nasiriyah Educational Hospital in Thi-Qar province for the period from November 2021 to March 2023. For each patient, a questionnaire was completed and demographic and clinical data were recorded.

-Diagnosis of cutaneous leishmaniasis:

-Direct Smear Method:

The direct stain method used to diagnose the cutaneous leishmaniasis, Tissue scrapings were taken from the margin of active cutaneous lesions by scalpel and blade, the sample spread on a clean glass slide and left to dry, methanol fixed, stained by Giemsa, and microscopically examined for the amastigote stage of the parasite which detected in macrophages in skin tissue (Al-Mayali,1998).

-HBA1C Test:

Blood samples were collected form 45 confirmed cutaneous leishmaniasis patients and placed in a size of (5 ml) in an EDTA tube for the purpose of measuring HBA1C for patients with cutaneous leishmaniasis.

-Statistical analysis:

The Data were analyzed by IBM SPSS Statistics program version 26 and P value equals or less than 0.05 considered statistically significant. Chi-square (χ^2) was used to compare between discrete independent variables distributed. The odds ratio OR was also used to determine whether diabetes exposure was a risk factor for some study outcome, and to compare the magnitude of different risk factors for that outcome. Microsoft® Excel 2010 was used to create the graphs.

Results:

The results of current study showed that 276 out of 315 (87.61%) were infected with cutaneous leishmaniasis by microscopic examination (Table1),(figure1):

Table [1]: The prevalence of cutaneous leishmaniasis by using the microscopic examination.

| Examined samples | Positive samples | % |
|------------------|------------------|-------|
| 315 | 276 | 87.61 |

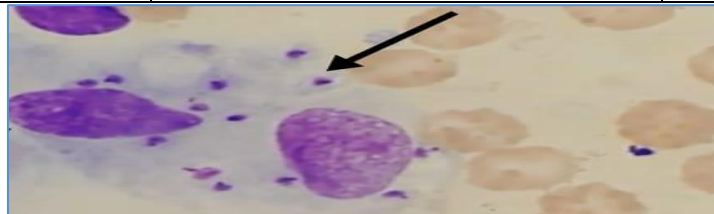


Figure (1): Amastigote stage of a cutaneous leishmaniasis parasite stained with Giemsa stain (100X).

No Significant differences ($P < 0.05$) were recorded in the prevalence of cutaneous leishmaniasis according to patient sex and the infected males 65.21% more than infected females 34.78%. Significant differences ($P < 0.05$) were recorded the prevalence of cutaneous leishmaniasis according to age groups, the highest prevalence on the age group (<20 year) 42.39% and the lowest prevalence on the age group (>40 year) 22.82%. (Table2).

Table [2]: Prevalence cutaneous leishmaniasis according to sex and age groups:

| No. | age group (year) | Males | % | Females | % | Total | % |
|-------|------------------|-------|-------|---------|-------|-------|-------|
| 1 | <20 | 70 | 20,36 | 47 | 17,02 | 117 | 42,39 |
| 2 | 20-40 | 69 | 20,00 | 27 | 9,78 | 96 | 34,78 |
| 3 | >40 | 41 | 14,80 | 22 | 7,97 | 63 | 22,82 |
| Total | | 180 | 60,21 | 96 | 34,78 | 276 | 100 |

χ^2 3.373 ^{Ns} P – value 0.185

The study recorded significant differences ($p \leq 0.05$) in prevalence of cutaneous leishmaniasis according to the location, the prevalence of cutaneous leishmaniasis in rural 66.30% more than the prevalence in urban 33.69%. (Table 3), (Figure 2).

Table [3]: The prevalence of cutaneous leishmaniasis according to location

| Location | No. of infected patients | % |
|----------|--------------------------|-------|
| Rural | 183 | 77,30 |
| Urban | 93 | 22,69 |
| Total | 276 | 100 |

$X^2:12.68$

P-value 0.001

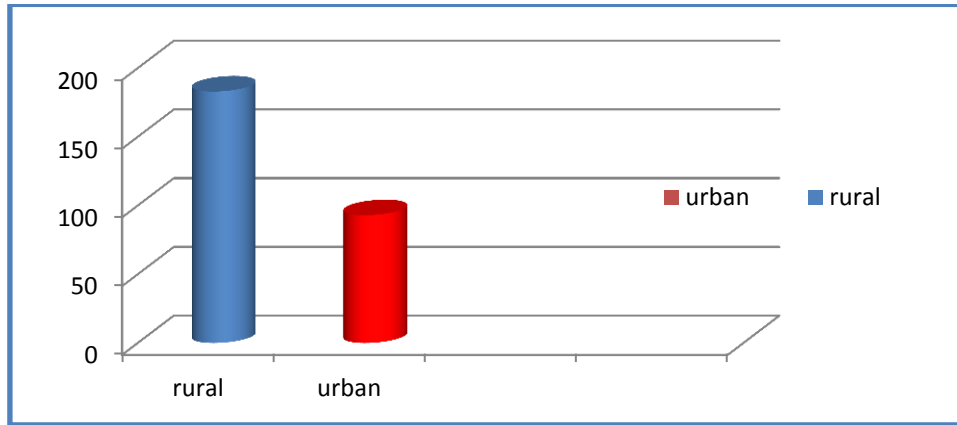
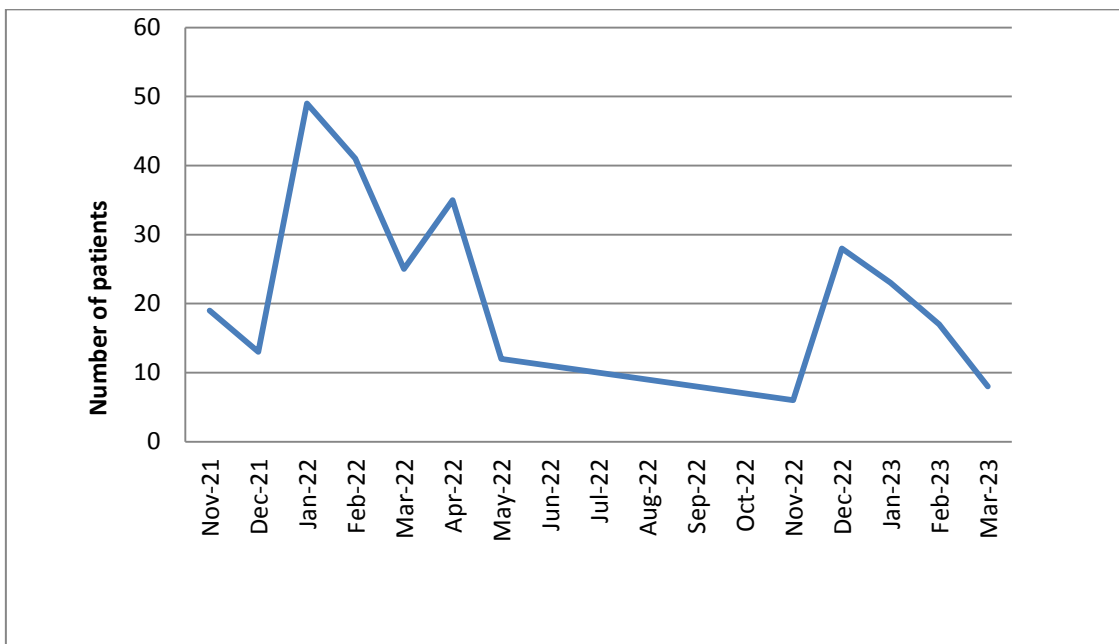


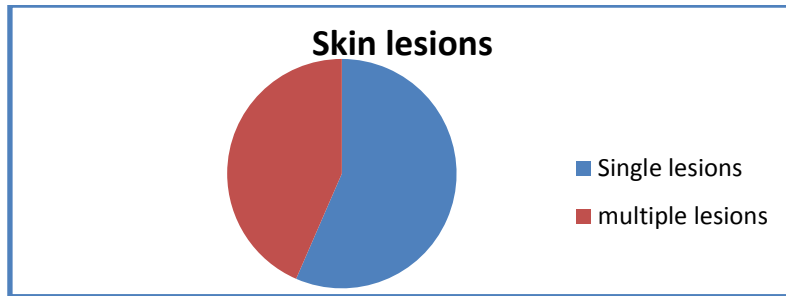
Figure (2): The prevalence of cutaneous leishmaniasis according to location.

In current study significant differences ($p < 0.05$) were recorded in prevalence of cutaneous leishmaniasis according to months of the year, the high prevalence recorded 17.75% in January 2022 and the lowest prevalence 2.89% in March 2023. (Figure 3).



Figure(3) The prevalence of cutaneous leishmaniasis according to months of the year

Significant differences ($p < 0.05$) were recorded in prevalence of cutaneous leishmaniasis according to single or multiple lesions, the single lesions showed high prevalence 56.52% while, the multiple lesions showed the lowest prevalence 43.47% (Figure 3).



Figure(4) The prevalence of cutaneous leishmaniasis according to single or multiple lesions.

A Significant differences ($p < 0.05$) were recorded in the prevalence of cutaneous leishmaniasis according to number of lesions, the high prevalence of cutaneous leishmaniasis 56.52% recorded in patients with one lesion and the lowest prevalence of cutaneous leishmaniasis 0.72 % recorded in patients with five lesions. Table 4.

Table [4]: The prevalence of cutaneous leishmaniasis according to number of lesions.

| No. of lesion | No. of infected patients | % |
|---------------|--------------------------|------------|
| ١ | ١٥٦ | ٥٦,٥٢ |
| ٢ | ٧٢ | ٢٦,٠٨ |
| ٣ | ٤٠ | ١٤,٤٩ |
| ٤ | ٦ | ٢,١٧ |
| ٥ | ٢ | ٠,٧٢ |
| Total | ٢٧٦ | ١٠٠ |

A Significant differences $p \leq 0.05$ were recorded in the prevalence of cutaneous leishmaniasis according to lesions site, the high prevalence 36.96 % reported in upper extremities and the lowest prevalence 14.13 % reported in face . (Table 5.)

Table[5]: The prevalence of cutaneous leishmaniasis according to lesions site

| Lesion site | No. patients with single lesions | % | No. of patients with multiple lesions | % | Total | % |
|-------------------|----------------------------------|------------|---------------------------------------|------------|------------|------------|
| upper extremities | ٥٣ | ٣٣,٩٧ | ٤٩ | ٤٠,٨ | ١٠٢ | ٣٦,٩٦ |
| Lower extremities | ٤٥ | ٢٨,٨٥ | ٣٧ | ٣٠,٨ | ٨٢ | ٢٩,٧١ |
| Face | ٣١ | ١٩,٨٧ | ٨ | ٦,٧ | ٣٩ | ١٤,١٣ |
| Trunk | ٢٧ | ١٧,٣١ | ٢٦ | ٢١,٧ | ٥٣ | ١٩,٢٠ |
| Total | ١٥٦ | ١٠٠ | ١٢٠ | ١٠٠ | ٢٧٦ | ١٠٠ |

$\chi^2: 9.994^{**}$

P.value :0.0186

The results showed that the highest prevalence was recorded in patients with dry lesions 48.55%, in compared with prevalence of patients with wet lesions 14.49%, while the prevalence of patients with dry and wet lesions was 36.95%. (Table 6).

Table [6]: The prevalence of cutaneous leishmaniasis according to lesion type.

| Lesion type | No. of infected patients | % |
|--------------|--------------------------|------------|
| Dry | ١٣٤ | ٤٨,٥٥ |
| Wet | ٤٠ | ١٤,٤٩ |
| Dry-Wet | ١٠٢ | ٣٦,٩٥ |
| Total | ٢٧٦ | ١٠٠ |

The results of current study reported 11 out of 45 cutaneous leishmaniasis patients were suffered from diabetes with prevalence 24.44%. A high association between diabetes and increase in the size of the skin lesions was recorded in current study, the prevalence of diabetic patients with large skin lesions 72.7% higher than the prevalence of diabetic patients with small skin lesions 27.3 %, while the prevalence of patients without diabetes who had small skin lesions 55.9 % higher than the prevalence of patients without diabetes who had large skin lesions 44.1 %. (Table7).

Table [7]: The relationship between diabetes and the development of skin lesions

| Patients with cutaneous leishmaniasis | Size of skin ulcer $\leq 2\text{cm}$ | % | Size of skin ulcer $> 2\text{cm}$ | % | The total | % |
|---------------------------------------|--------------------------------------|--------------|-----------------------------------|--------------|-----------|------------|
| Diabetic patients | 8 | 72.7 | 3 | 27.3 | 11 | 100 |
| Non-diabetic patients | 15 | 44.1 | 19 | 55.9 | 34 | 100 |
| The total | 22 | 48.89 | 23 | 51.11 | 45 | 100 |

OR:3.378

$\chi^2:2.722$

CI95%: 0.762-14.982

P value 0.098 NS

The current study reported an association between diabetes and increased the number of cutaneous skin lesions. The prevalence of cutaneous leishmaniasis patients with diabetes who suffered from multiple skin lesions was 54.5 % higher than the prevalence of cutaneous leishmaniasis patients with diabetes who had a single skin lesions 45.5%, While, the prevalence of cutaneous leishmaniasis patients without diabetes and had a single skin lesion 91.2% was higher than the prevalence of cutaneous leishmaniasis patients without diabetes and had multiple skin lesions, 8.8 % (Table, 8).

Table [8]: The relationship between diabetes and the number of skin lesions:

| cutaneous leishmaniasis Patients | Single lesion | % | Multiple lesion | % | The total | % |
|----------------------------------|---------------|-------|-----------------|-------|-----------|-----|
| With diabetic | 5 | 45.5 | 6 | 54.5 | 11 | 100 |
| Without diabetic | 31 | 91.2 | 3 | 8.8 | 34 | 100 |
| Total | 36 | 80.00 | 9 | 20.00 | 45 | 100 |

$\chi^2: 10.109$ **P value: 0.0009****

The results of the current study showed an association between diabetes and non-response to treatment of cutaneous leishmaniasis patients, the prevalence of cutaneous leishmaniasis patients with diabetes who did not respond to treatment was 63.6 % higher than the prevalence of cutaneous leishmaniasis patients with diabetes who responded to treatment 36.4%, while the prevalence of cutaneous leishmaniasis patients without diabetes who responded to treatment was 91.2% higher than the prevalence of cutaneous leishmaniasis patients and without diabetes who did not respond to treatment 8.8 % . (Table, 9).

Table [9]: The relationship between diabetes and response to treatment:

| Cutaneous leishmaniasis patients | Response to treatment | % | Not respond to treatment | % | Total | % |
|----------------------------------|-----------------------|-------|--------------------------|-------|-------|-----|
| With diabetic | 4 | 36.4 | 7 | 63.6 | 11 | 100 |
| Without diabetic | 31 | 91.2 | 3 | 8.8 | 34 | 100 |
| Total | 35 | 77.78 | 10 | 22.22 | 45 | 100 |

$\chi^2 14.447$ **P value 0.0001****

Discussion:

The results of current study showed that 276 out of 315 (87.61%) were suspected infected with cutaneous leishmaniasis by microscopic examination. The study of [8] in some provinces of Iraq, recorded prevalence of cutaneous leishmaniasis 15.1% in Diwanayah, Wasit 14.5%, Najaf 13.6%, Thi- Qar 13.1%, Basra 11.5%, , Baghdad 11.2%, Diyala 10.8%, Salah al-

Din 10.3%. The study of [9] recorded 106 people were infected with cutaneous leishmaniasis in Al-Najaf Al-Ashraf province. The study of [10] in Baghdad province indicated that 54% cases in Rusafa and 80% cases in Al-Karkh in 2019. were infected with cutaneous leishmaniasis within the age group of 3-71 years. The study of [11] showed that among 4276 samples collected from people who visited Al-Diwaniyah Teaching Hospital after suffering from a skin infection, there were 1489 confirmed cases of cutaneous leishmaniasis during 2018.

Significant differences ($P \leq 0.05$) were recorded in the prevalence of cutaneous leishmaniasis according to patient sex and the infected males 65.21% more than infected females 34.78%. The reason may be that the male body parts are most exposed to sandfly bites, due they may play and sleep most time with short cloths, also coming out to play from their houses. In addition, the insect activity increases in outside the houses. Significant differences ($P < 0.05$) were recorded the prevalence of cutaneous leishmaniasis according to age groups, the highest prevalence on the age group (<20 year) 42.39% and the lowest prevalence on the age group (>40 year) 22.82%. That most lesions occurred within small age groups, this can be explained as result to previous exposure of the infection which give permanent immunity for individuals or due the immunity development with the age, this means that the immune is a weak in early age. The study of [12] noted in Thi- Qar province that the number of infected females was 37 (75.5%) which was higher than the number of infected males 49 (64.5%), the highest prevalence was recorded within the age group (81-90) years 100%, and the lowest prevalence was within the age group (51-60) years 62.5%, while no infections were recorded in the age groups (61-70) and (71-80) years.

The study of [13] indicated in Thi- Qar province that there was a significant difference between prevalence of cutaneous leishmaniasis of males 66.4% and females 33.6%. The highest prevalence of cutaneous leishmaniasis within the age group under ten years. A study of [14] in Thi- Qar province indicated that the highest prevalence of cutaneous leishmaniasis was in males 60.8% when compared to females 39.2%, and the highest prevalence in the age group of 10 years was 29.6% when compared to the age group 70-79 years 0.8%.

The study recorded significant differences ($p < 0.05$) in prevalence of *cutaneous leishmaniasis* according to the location, the prevalence of cutaneous leishmaniasis in rural 66.30% more than the prevalence in urban 33.69%. The study of [12] in Thi-Qar province indicated the highest prevalence of cutaneous leishmaniasis in rural 73.2%, while the prevalence of people living in urban areas 65.2%, the study of [13] showed that city centers had the highest prevalence of *cutaneous leishmaniasis* compared to the countryside. The study of [14] showed that the highest prevalence of cutaneous leishmaniasis was in the Nasiriyah district 31.2%, while the lowest prevalence of *cutaneous leishmaniasis* was 0.8% in the Al-Fadliyah, Al-Fajr, and Al-Tar districts and the highest prevalence was 63.2% in urban areas, while the lowest prevalence was 36.8% for rural areas. The results of [12] in the central and southern provinces showed that the prevalence of *cutaneous leishmaniasis* of people in rural areas was 61.77% over the prevalence of people infected in urban areas 38.22%.

The incubation period for leishmaniasis plays an important role in fluctuating prevalence due to the effect of climate on the parasite's vector [2].

In current study significant differences ($p \leq 0.05$) were recorded in prevalence of cutaneous leishmaniasis according to months of the year, the high prevalence recorded 17.75% in January 2022 and the lowest prevalence 2.89% in March 2023. The temperature effects at sandfly activity. A temperature and an incubation period of *Leishmania* spp. Play a role in the fluctuation of the infection percentages. A study of [13] indicated that the highest prevalence of cutaneous leishmaniasis was recorded in December 29.6%, and no infections were recorded in September and October, and a study of [15] in Thi-Qar province showed that infections gradually increase during October and November, decrease in July, and disappear in August. A study of [14] showed that the highest prevalence of cutaneous leishmaniasis was 37.6% in January, and no infection was recorded in June, July, and August. The study of [16] in Maysan province recorded that the highest prevalence of cutaneous leishmaniasis were in the months of January 31.45% and February 33.87%.

A Significant differences $p \leq 0.05$ were recorded in the prevalence of cutaneous leishmaniasis according to number of lesions, the high prevalence of cutaneous leishmaniasis 56.52% recorded in patients with one lesion and the lowest prevalence of cutaneous leishmaniasis 0.72 % recorded in patients with five lesions. The results of [12] recorded in Thi-Qar province the high prevalence of cutaneous leishmaniasis in a patient with 12 lesion 100%, while the lowest prevalence of cutaneous leishmaniasis was 33.3% in patients with 7 lesions. A study of [13] showed that the prevalence of single lesion 55.47% (males 35.22% and females 20.25%), while the prevalence of multiple lesions 44.53% (males 31.17% and females 13.36%), there was a non-significant difference ($P > 0.05$) between single, double and multiple lesions: 3, 4, 5, and more than 5. A study of [14] recorded significant differences in the number of lesions, the prevalence of single lesion 45.6%, two lesions 24.8%, three lesions 20% and more than three lesions 9.6%.

The highest prevalence of single lesion was recorded, followed by double and multiple lesions. This may be due to the fact that the female sand fly has usually taken a person's blood once, so most infections appear as single lesion, while multiple lesions may occur as a result of insects biting the same person more than once, or due to the density of sand flies in local areas. Sandflies bite differently at each feeding stage, or the abundance of infected sandflies may play a role [17]. Moreover, *L. tropica* is usually associated with single lesions, while *L. major* infections are associated with multiple lesions [18,19].

A study of [13] recorded that the single lesions showing the highest prevalence. The study of [20] in Al-Qadisiyah province showed that most patients were infected with single lesion, while a study of [21] recorded that the single lesion had the highest prevalence 22.9%. The study of [22] in Mesan province recorded that the prevalence of single lesion was 53.91% and the prevalence of multiple lesions was 11.11%. The multiple lesions due to long periods of exposure to repeated feeding attempts of sand flies, and the high density of sand flies in this region [23].

The results of current study reported 11 out of 45 cutaneous leishmaniasis patients were suffered from diabetes with prevalence 24.44%. A high association between diabetes and increase in the size of the skin lesions was recorded in current study, the prevalence of diabetic patients with large skin lesions 17.78% higher than the prevalence of diabetic patients with small skin lesions 6.67%, while the prevalence of patients without diabetes who had small skin lesions 42.22% higher than the prevalence of patients without diabetes who had large skin lesions 33.33%. A study of [24] indicated the large size of cutaneous leishmaniasis lesions in patients with diabetes, the high levels of interferon-gamma and tumor necrosis factor in response to Leishmania antigen in diabetic patients.

The current study reported an association between diabetes and increased the number of cutaneous skin lesions. The prevalence of cutaneous leishmaniasis patients with diabetes who suffered from multiple skin lesions was 13.33% higher than the prevalence of cutaneous leishmaniasis patients with diabetes who had a single skin lesions 11.11%, While, the prevalence of cutaneous leishmaniasis patients without diabetes and had a single skin lesion 68.89% was higher than the prevalence of cutaneous leishmaniasis patients without diabetes and had multiple skin lesions, 6.67%. The diabetes impairs the function of neutrophils and monocytes in cutaneous leishmaniasis patients which led to increase in the number of skin lesions in cutaneous leishmaniasis patients with diabetes, the high level of blood glucose increase the attraction of the sand fly to feed more than once [24].

The results of the current study showed an association between diabetes and non-response to treatment of cutaneous leishmaniasis patients, the prevalence of cutaneous leishmaniasis patients with diabetes who did not respond to treatment was 15.56% higher than the prevalence of cutaneous leishmaniasis patients with diabetes who responded to treatment 8.89%, while the prevalence of cutaneous leishmaniasis patients without diabetes who responded to treatment was 68.89% higher than the prevalence of cutaneous leishmaniasis patients and without diabetes who did not respond to treatment 6.67%. The cutaneous leishmaniasis in diabetic patients stimulates the production of pro-inflammatory cytokines and weakens the immune response to treatment[24]. The results of current study demonstrated a significant relationship between diabetes and cutaneous leishmaniasis in distinct risk determinants. Also, the study showed that the diabetes increased the severity of active cutaneous leishmaniasis.

Conclusions:

- 1-** Males, children and adolescents, and rural areas are most susceptible to infection with cutaneous leishmaniasis in Thi Qar province.
- 2-** Single lesions (one ulcer) are the most common, and the upper extremities are the areas of the body most exposed to injuries, followed by the lower extremities, then the face.
- 3-** Diabetes is a risk factor associated with an increase in the size and number of skin lesions, and an increased possibility of secondary bacterial infection accompanying the skin lesions with the cutaneous Leishmania parasite.

REFERENCE:

1. Uzun, S., Gürel, M. S., Durdu, M., Akyol, M., Fettahlioğlu Karaman, B., Aksoy, M., ... & Harman, M. (2018). Clinical practice guidelines for the diagnosis and treatment of cutaneous leishmaniasis in Turkey. *International journal of dermatology*, 57(8), 973-982.
2. Alsaad, R. K. A., & Kawan, M. H. (2021). An epidemiological study of cutaneous leishmaniasis in human and dogs. *Annals of parasitology*, 67(3), 417-433. <https://doi.org/10.17420/ap6703.355>.
3. Gürel, M. S., Tekin, B., & Uzun, S. (2020). Cutaneous leishmaniasis: A great imitator. *Clinics in dermatology*, 38(2), 140-151.
4. Bilgic-Temel, A., Murrell, D. F. and Uzun, S. (2019). 'Cutaneous leishmaniasis: A neglected disfiguring disease for women.
5. Sadlova, J., Myskova, J., Lestinova, T., Votypka, J., Yeo, M., & Volf, P. (2017). *Leishmania donovani* development in *Phlebotomus argentipes*: comparison of promastigote- and amastigote-initiated infections. *Parasitology*, 144(4), 403-410. <https://doi.org/10.1017/S0031182016002067>.
6. Cecílio, P., Cordeiro-da-Silva, A., & Oliveira, F. (2022). Sand flies: Basic information on the vectors of leishmaniasis and their interactions with *Leishmania* parasites. *Communications biology*, 5(1), 305. <https://doi.org/10.1038/s42003-022-03240-z>.
7. Reimão, J. Q., Coser, E. M., Lee, M. R., & Coelho, A. C. (2020). Laboratory Diagnosis of Cutaneous and Visceral Leishmaniasis: Current and Future Methods. *Microorganisms*, 8(11), 1632. <https://doi.org/10.3390/microorganisms8111632>.
8. Ali, M. A., Khamesipour, A., Rahi, A. A., Mohebbali, M., Akhavan, A., Firooz, A. and Keshavarz, H. V. (2018). 'Epidemiological study of leishmaniasis in some Iraqi provinces', *J. Men. Heal.*, 14(4): 18-24. doi: 10.22374/1875-6859.14.4.4.
9. Al-Alawi, H . M (2020) . Study of some epidemiological, biochemical and molecular aspects of the *Leishmania* parasite Dermatology in Najaf Al-Ashraf province , MCQ . Thesis . College of Education for Girls . University of Kufa . 124 PP.
10. Turki, W. Q., Reda, J. I. A. ., Abbas, R. H. ., & Rdaali, A. R. R. F. . (2022). Survey Study of Cutaneous Leishmaniasis in Baghdad . *Ibn AL-Haitham Journal For Pure and Applied Sciences*, 35(3), 1-4. <https://doi.org/10.30526/35.3.2740>.
11. Al-Waaly, A.B.M.; Shubber, H.W.K. (2020). Epidemiological study of cutaneous leishmaniasis in Al-Diwaniyah Province, Iraq. *Eurasia. J .Bio.Sci.*, 14, 269-273 pp.

- 12. Al-Mosawi, N. A.J. AK. (2015).** Investigate of Cutaneous Leishmaniasis and knowledge of the role heat shock protein HSP70 in the immune response in the province of Thi Qar . M.Sc. College of Education for pure science. University of Thi-Qar. 90 pp.
- 13. Flaih, M. H. (2020).** Epidemiology and Molecular study of Leishmania tropica Isolated from Cutaneous Lesions in Thi-Qar Province, Iraq. Ph.D. Thesis. College of Education for pure science. University of Thi-Qar. 165 pp.
- 14.Musa, F. G. (2022).** Detection of cutaneous Leishmania parasite in skin tissue using immunohistochemistry technique.MCQ. Thesis. College of Education for pure science. University of Thi-Qar. 215 PP.
- 15.Atshan, A. M. (2014).** Epidemiological study for distribution of Cutaneous and Viseral Leishmaniasis in Thi-Qar province and test efficiency some pesticides on the insect vector. M.Sc. Thesis. College of Science.University of Thi-Qar. 106.
- 16.Abul-Doanej, H. A. I. (2014).** Study of Epidemiological aspects for Leishmaniasis and diagnosis of the Parasite by using NestedKinetoplast Minicircle DNA-PCR technique In the Province of Maysan-Iraq. M.Sc.College of Education for Pure Science. University of Basrah. 93 pp.
- 17.Rahmanian, P., Bryson, A. L., Binnicker, M. J., Pritt, B. S., & Patel, R. (2018).** Syndromic panel-based testing in clinical microbiology. Clinical microbiology reviews, 31(1), e00024-17.
- 18.Al-Mafraji, K. H., Al-Rubaey, M. G. and Alkaisy, K. K. (2008).** ‘ClinicoEpidemiological Study of Cutaneous Leishmaniasis in Al-Yarmouk Teaching Hospital’, Iraqi J. Comm. Med., 3:194–197.
- 19.Al-Difaie, R. S. (2013).** Prevalence of Cutaneous Leishmaniasis in ALQadissia province and the evaluation of treatment response by pentostam with RT-PCR. Wasit Uni. Coll. Sci.
- 20.Al-Mayali, H. M. (1998)** . Evaluation and use of some immunological tests in an epidemiological study of leishmaniasis in Al-Qadisiyah province , Ph.D. Thesis. College of Education for pure science. University of Al-Qadisiyah. 196 pp.
- 21.Al-Hassani, M. K. K. T. (2016).** Epidemiological, Molecular and Morphological Identification of cutaneous leishmaniasis and, It’s insect vectors in Eastern Al-Hamzah district,AlQadisiya province. College. Education. University of AL-Qadisiya .101pp.
- 22.Al-Lami S.(2021).** Epidemiological and diagnostic and genetics study for leishmania parasite in Misan government. M.Sc. College of Education for pure science. University of Thi-Qar. 76 pp .
- 23.Bachi, F., Icheboudene, K., Benzitouni, A., Taharboucht, Z., & Zemmouri, M. (2019).** Épidémiologie de la leishmaniose cutanée en Algérie à travers la caractérisation moléculaire. Bull Soc Pathol Exot, 112, 147-52.

24.Lago, A. S., Lima, F. R., Carvalho, A. M., Sampaio, C., Lago, N., Guimarães, L. H., ... & Carvalho, E. M. (2020, December). Diabetes modifies the clinic presentation of cutaneous leishmaniasis. In *Open Forum Infectious Diseases* (Vol. 7, No. 12, p. ofaa491). US: Oxford University Press.