Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

Bio Changes Related with Cutaneous Leishmaniasis Patients in Babil Governorate.

Aseel Zuhair Sherba[1], Malak Majid Almusawi[2]

[1]Ph.D-Student., Part of Thesis, Department of Biology, Faculity of Education for girls, University of Kufa, Najaf, Iraq.

[2]Advisor., Part of Thesis., Department of Biology, Faculity of Education for girls, University of Kufa, Najaf, Iraq.

KEYWORDS
amastigotes,
lesion,
leishmaniasis,
anthroponotic.

ABSTRACT:
The study was performed to evaluate the histological changes in ulcers of infected patients with cutaneous leishmaniasis in Babil governorate/ Iraq. The study was performed in El-Sadeq hospital, Al-kifel hospital, Musaib hospital and Alexandria hospital within Governorate of Babil from 1/9/2021 to 1/4/2022. In our study we used full thickness biopsy stained with (H&E) and direct smear stained with Geimsa stain technique taken from each ulcer of 22 patients infected with CL. We could identify amastigote stage of the parasite in 8 of 22 patient (36%) by full thickness biopsy and (H&E) stain and in 10 of the 22 (45%) infected patients using direct smear stained with Geimsa stain. We could identify amastigote in one slide in the epidermis which is considered a rare finding. We could identify a heavy inflammatory cell in filteration in the superficial dermis which is considered a diagnostic feature by some authors in the absence of amastigotes in the lesion. Various studies were done to define the best technique to obtain the outmost results to identify the parasite and characteristic features in a smear or biopsy of the lesion.

INTRODUCTION

Leishmaniasis is a vector-borne parasitic disease. It is triggered by obligate intracellular protozoa of the Leishmania genus and transmitted to humans by the bite of female phlebotomine sand flies (Grayson,2012) In Iraq two species are present , *L.tropica* which is the cause of anthroponotic cutaneous leishmaniasis and *L.major* which cause the zoonotic cutaneous leishmaniasis. The ACL is found in urban and sub urban region and ZCL found in rural areas. (WHO,2003). The total incidence rate of cutaneous leishmaniasis in Iraq varies from 2.3/100,000 to 45.5/100,000 (WHO 2003). There had been two peaks of CL. occurred during last thirty years ,the first in 1992,the infection rate reached 8779 infected person (Korzeniewski, 2005). The second peak happened in 2015 where the reported cases reached 4000 patient(WHO2021).Persons who have cutaneous leishmaniasis have one or more sores on their skin. The sores can change in size and appearance over time. They often end up looking somewhat like a volcano, with a raised edge and central crater. A scab covers some sores. The sores can be painless or painful. Some people have swollen glands near the sores (for example, in the armpit if the sores are on the arm or hand)(Arfan & Rahman,2006). The diagnostic methods available are mostly based on clinical and epidemiological evidence and parasite detection, no single laboratory method has been accepted as a gold standard for diagnosis CL. Parasitological tests of a skin biopsy specimen are not always conclusive in patients with a clinical diagnosis of CL (Schalling&Oskam ,2002). Leishmaniasis is diagnosed by demonstrating the presence of Leishmania amastigotes in clinical specimens using direct microscopic examination or molecular analysis based on nuclear or kinetoplast DNA amplification. Amastigotes are round and have a diameter of 1 to 4 µm and a characteristic kinetoplast which appears as rod-shaped structure (Abadías *et al*.,2021) Diagnosis is based on clinical appearance and evolution of sore, history of visit to endemic areas but confirmation is through demonstration of the amastigote parasite, Leishmania bodies in sores.(Burton,2009). Histological examination of skin biopsy requires specialized setup and processing unit while

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

microscopic examination of skin smears is easy to perform, cost effective and can be done in outdoor clinic as it does not require specialized equipment (Anderson,1996).

MATERIAL AND METHODS

22 patients were selected of the diagnosed patients by specialized Dermatologist physician and made a full thickness biopsy section for histopathological study, and a slide smear for the direct light microscope examination . the skin area was thoroughly cleaned with ethyl alcohol at a concentration of 70%, then left to dry. Injection of 0.2-0.1 mL of sterile saline (sterile saline) subcutaneously at the pink edge of the skin around the ulcer and after withdrawing the syringe, a drop of aspirate was taken from the area and placed on a clean glass slide and made a swab fixed with ethyl alcohol at a concentration of 95% and dyed with Geimsa stain to confirm the presence of the amastigote stage of Leishmania parasites Adini *et al*(1998).

RESULTS DIRECT SMEAR

Aspirate smears of 10 slides were positive for amastigote of cutaneous leishmaniasis of 22 slides stained with geimsa stain with45% figure (1)

•

HISTOPATHOLOGICAL FINDINGS:

Full thickness biopsy was positive for identifying amastigotes in the dermis in 8 of 22 slides of cutaneous leishmaniasis with 36.3% using H&E stain (Figure2). In one slide amastigote was identified in epidermis within necrotic cells and hyperplasia of epidermis Figure (3).

The results of full thickness biopsy of cutaneous leishmaniasis were as follows**;**

EPIDERMAL CHANGES;

- Epidermal hyperplasia figure (4)
- Epidermal hyperplasia with hyperkeratinization squamous cell activity figure (5, 6).
- Necrosis of epithelial cells figure(7, 8, 9)
- Fibrosis areas in the Epidermis (10)
- Inflammatory cells infilteration (4, 7, 8, -9) Dermal changes;
- Inflamatory cells infiltration mostly mononuclear cells in the dermis. figure (2, 4, 9, 11)
- Fibrous tissue deposition. Figure (2, 12)
- Granuloma like features. Figure (13, 14)

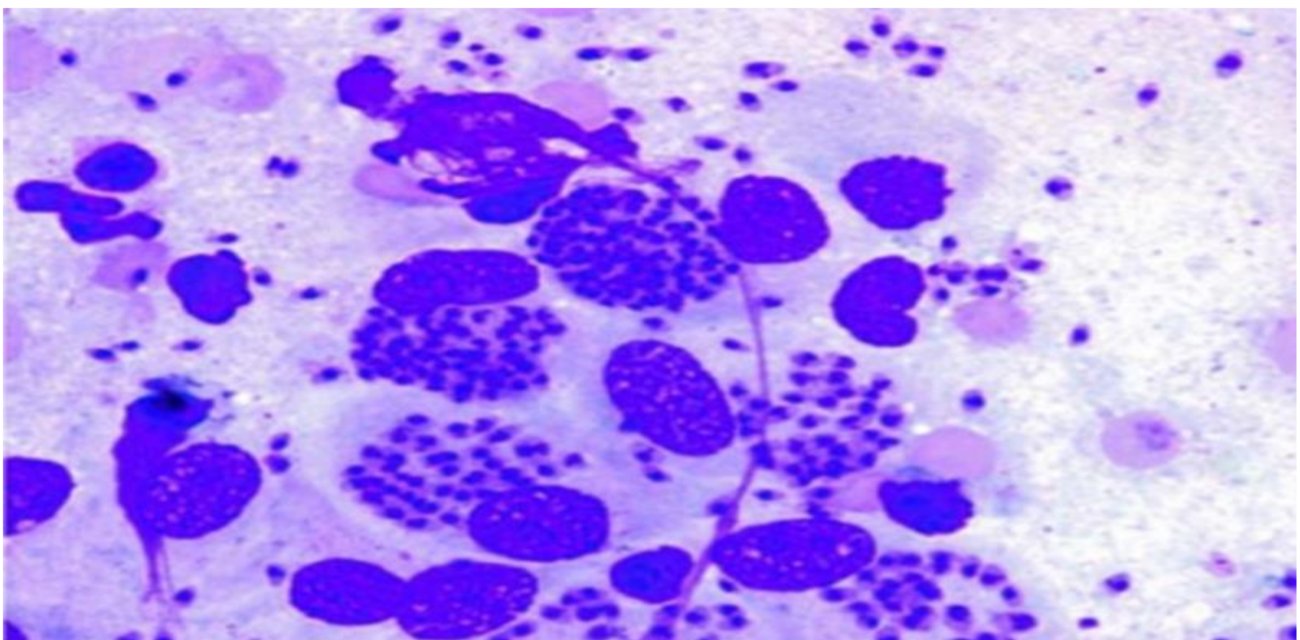


Figure (1) Extra cellular and intracellular amastigotes mostly mononuclear cells.

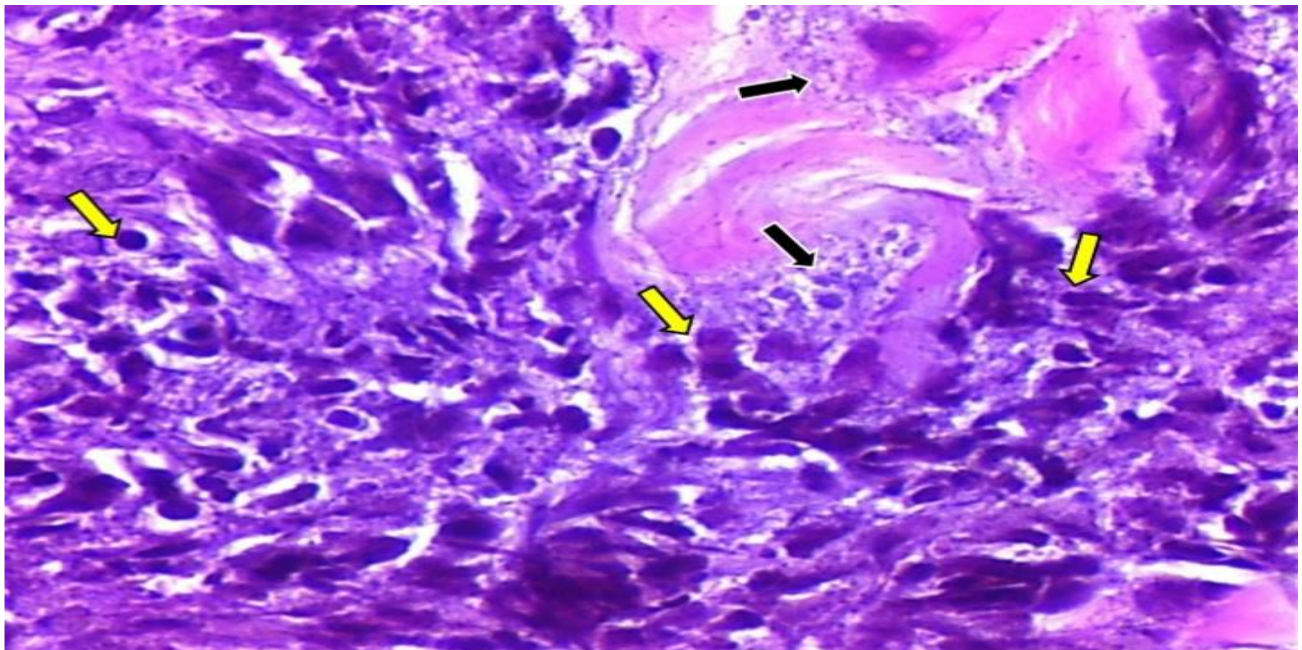


Figure (2) Leishmania amastigotes (black arrow) were observed in dermis layer of infected skin within the inflammatory cells' aggregation (yellow arrow) with presence of fibrous tissue in the affected area. H&E. 400x.

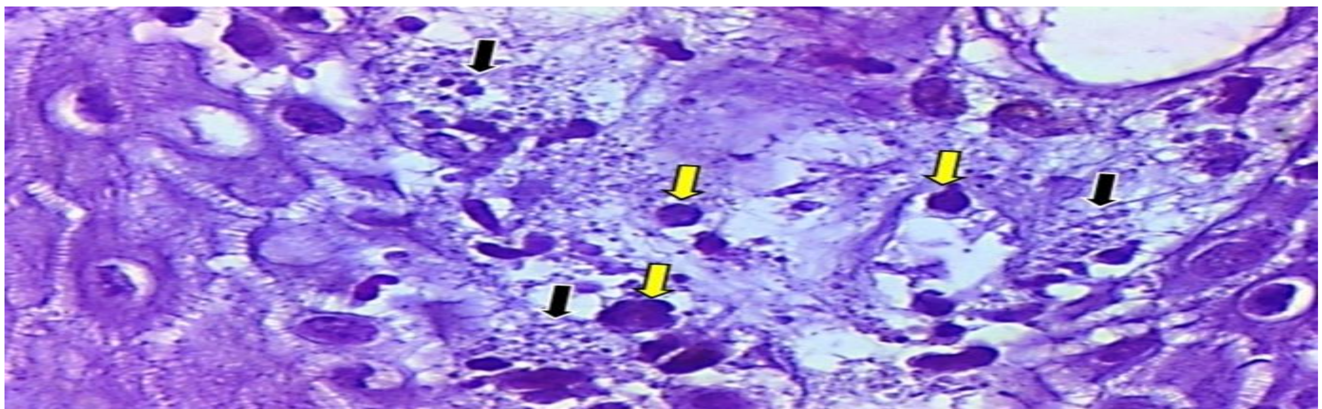
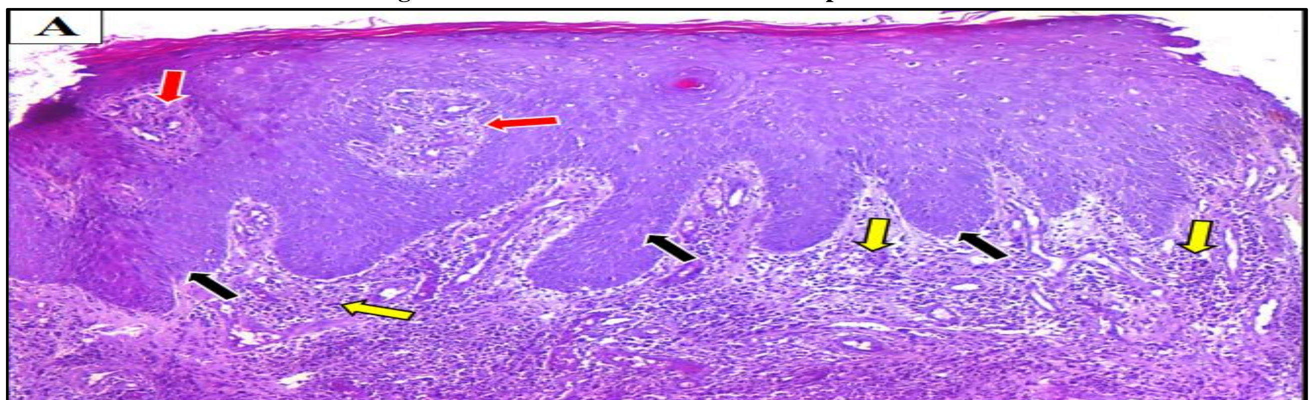


Figure (3): Photomicrograph of leishmania infected skin Necrosis of epithelial cells that formed spaces in epidermis with presence of inflammatory cells (yellow arrow) mostly mononuclear in these spaces were observed. Note the Leishmania amastigotes were observed in necrotic cells spaces of affected areas. H&E. 400x.



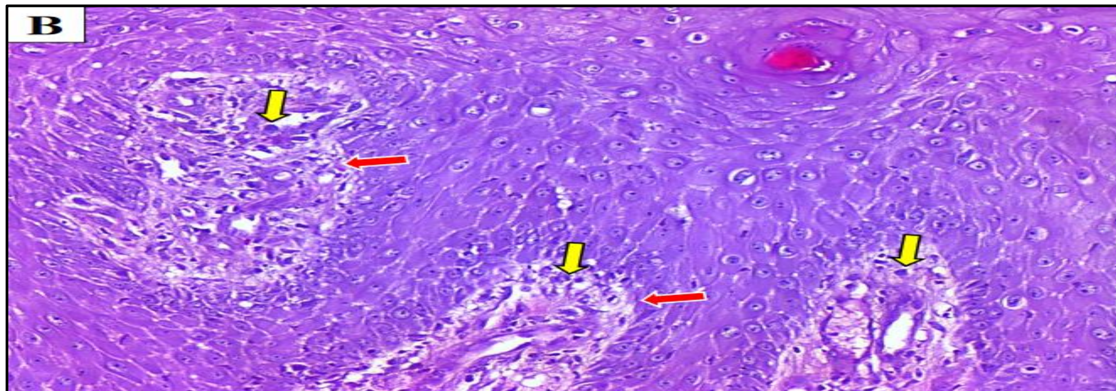


Figure (4)A&B/ Hyperplasia of squamous epithelial cells of epidermis layer that extended as projection toward dermis layer led to thickening of epidermis layer (black arrow) of infected skin. Also, necrosis of epithelial cells that formed spaces (red arrow) in epidermis with presence of inflammatory cells (yellow arrow) in these spaces were observed. Massive infiltration of inflammatory cells (yellow arrow) mostly mononuclear cells in dermis layer of infected skin. H&E. A: 40x and B: 100x.

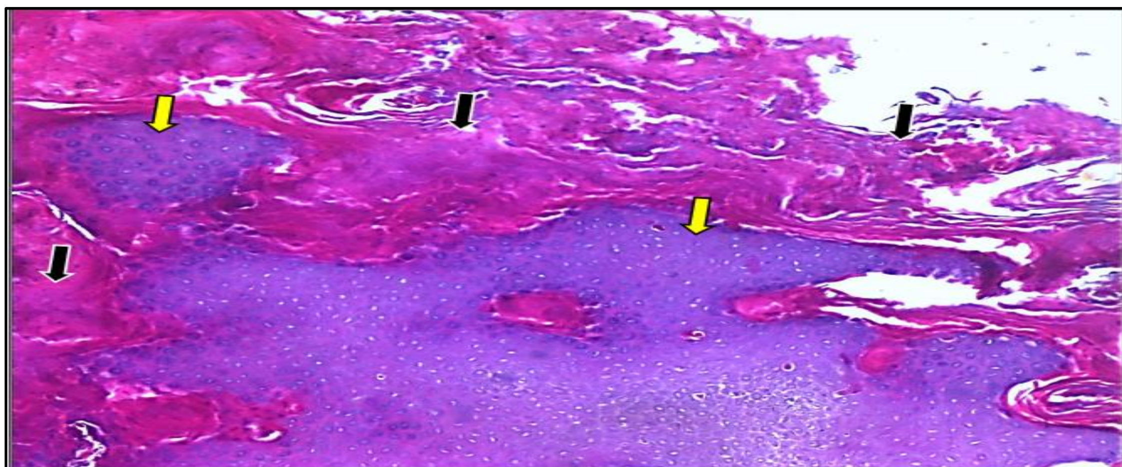
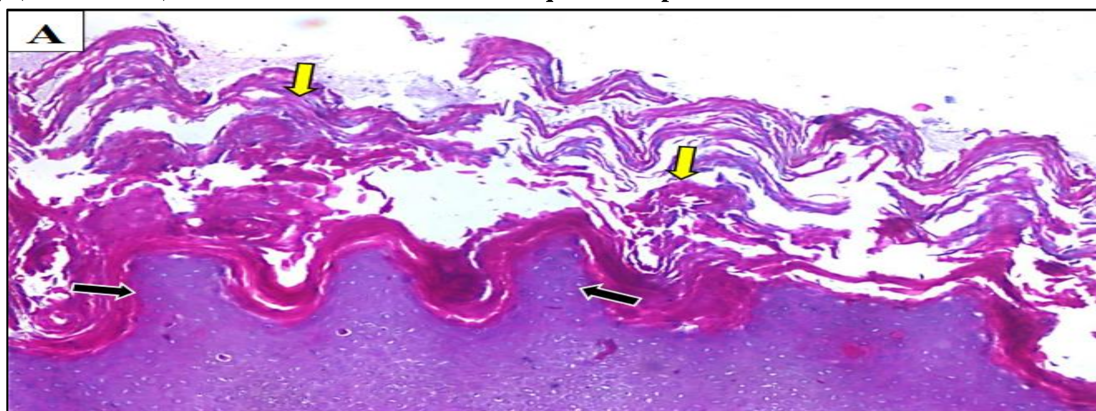
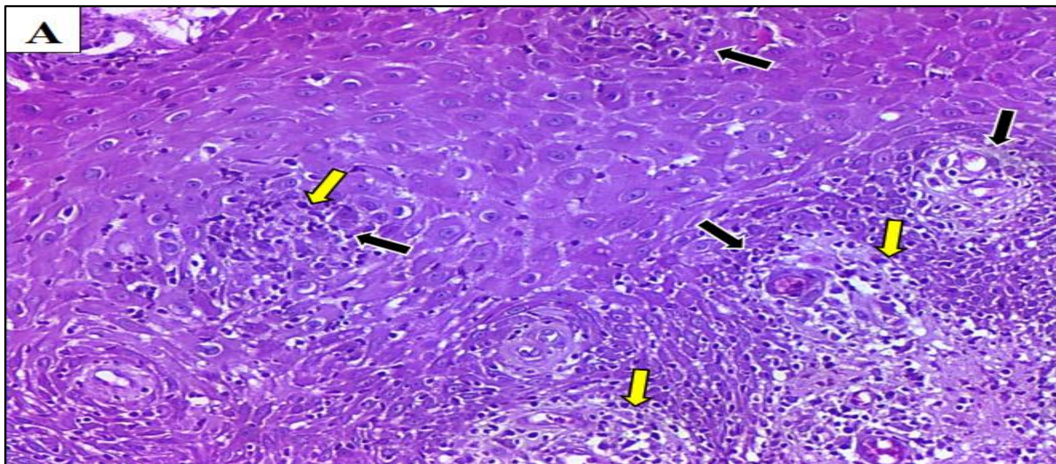


Figure (5): Photomicrograph of leishmania infected skin Hyperplasia of squamous epithelial cells of epidermis layer that formed masses (red arrow) above skin layers, also hyper-keratinization squamous epithelial cells activity (black arrow) was observed that surrounded squamous epithelial cells masses was observed. H&E. 40x.

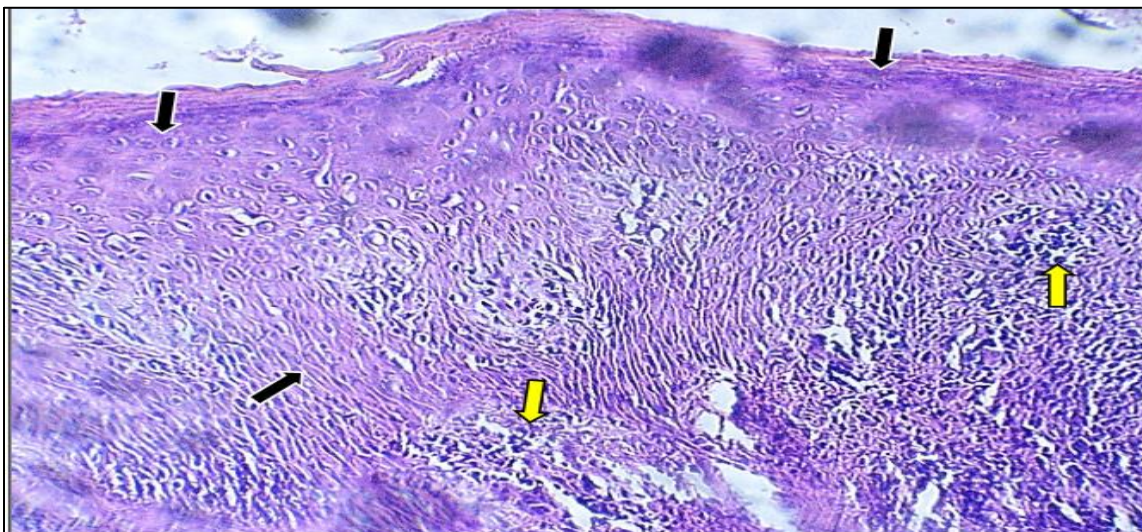


Figure(6): Photomicrograph of leishmania infected skin . Hyperplasia of squamous epithelial cells of epidermis layer, where the edge of epidermis was irregular in shape (black arrow) with presence of Hyper-keratinization squamous epithelial cells activity (yellow arrow) was observed above the affected epidermis.

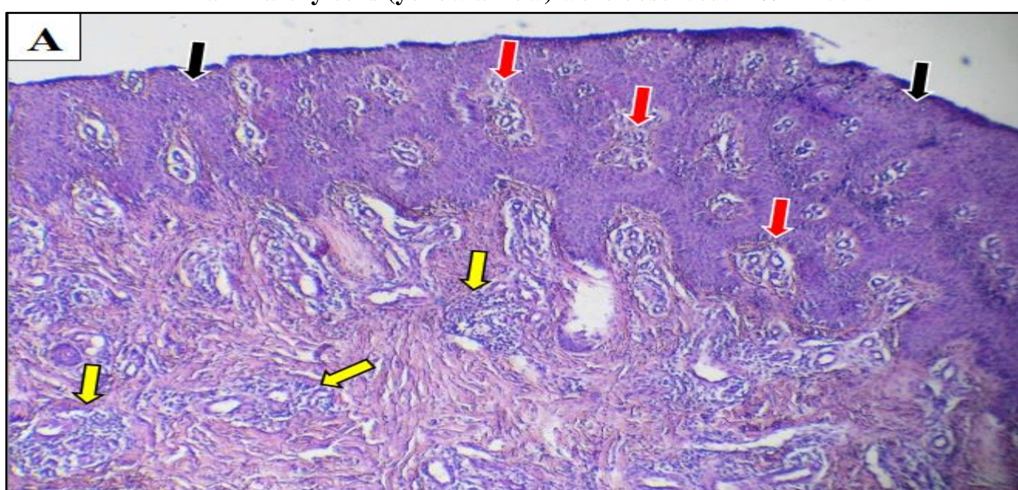
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727



Figure(7) Necrosis of epithelial cells that formed spaces (black arrow) in epidermis with presence of inflammatory cells (yellow arrow) in these spaces were observed



Figure(8) Hyperplasia of squamous epithelial cells of epidermis layer led to thickening of epidermis layer (black arrow) of infected skin. Also, necrosis of epithelial cells that formed spaces in epidermis that filled with inflammatory cells (yellow arrow) were observed. H&E. 100x.



Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

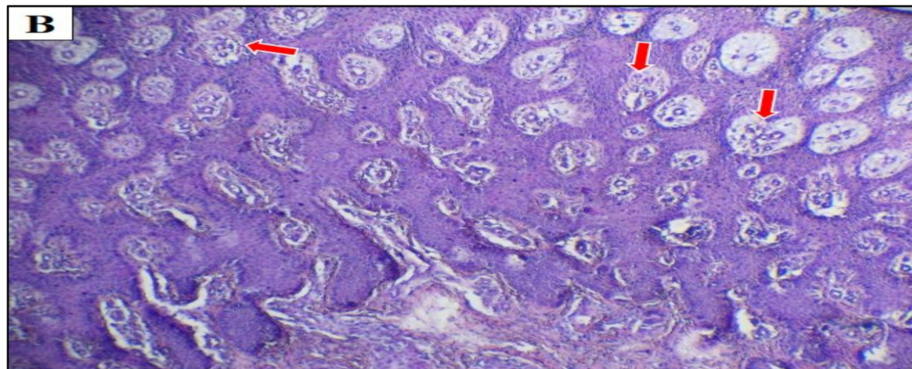


Figure (9): Photomicrograph of leishmania infected skin

A / Hyperplasia of squamous epithelial cells of epidermis layer led to thickening of epidermis layer (black arrow) of infected skin. Also, necrosis of epithelial cells formed spaces (red arrow) in epidermis that filled with inflammatory cells were observed. Infiltration of inflammatory cells (yellow arrow) that aggregated as nests in dermis layer. B/ Note the necrotic cells spaces (red arrow) occupied most of epidermis layer. H&E. A: 40x and B: 100x.

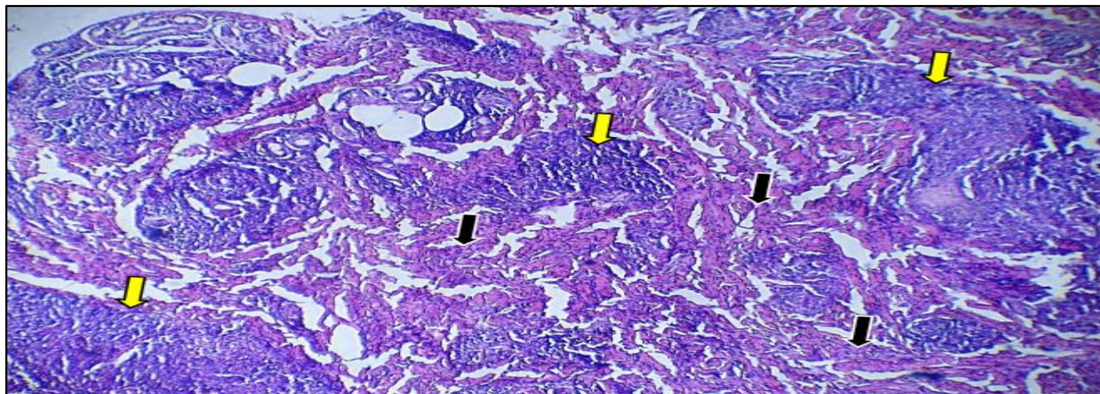
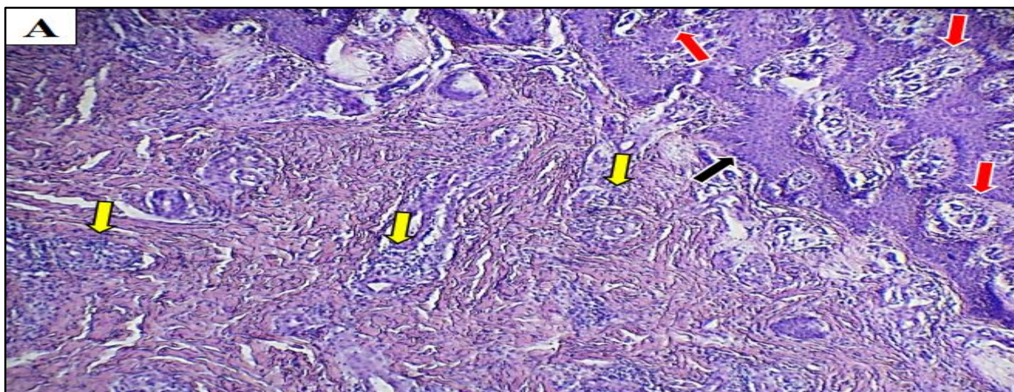


Figure (10): Photomicrograph of skin of leishmania infected skin .

Hyperplasia of squamous epithelial cells of epidermis layer that formed masses (yellow arrow) above skin layers that surrounded by fibrous tissue (black arrow). H&E. 40x.



Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

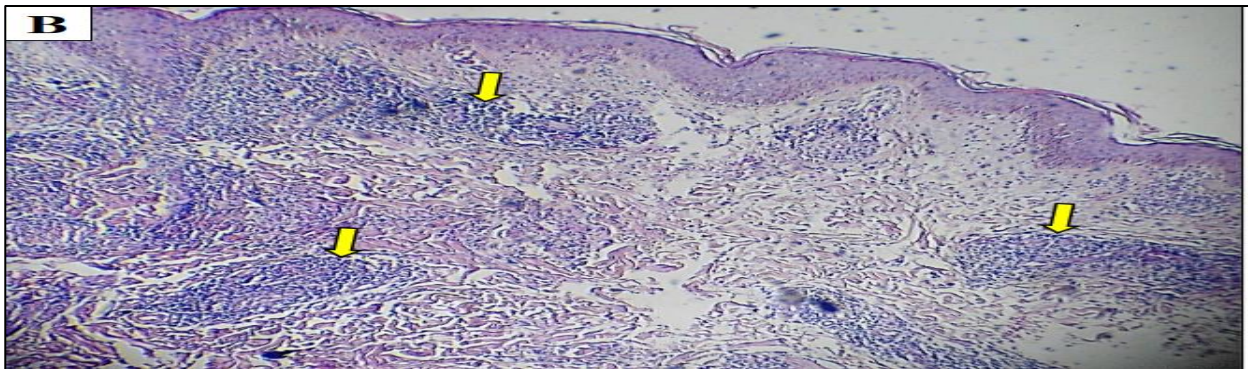
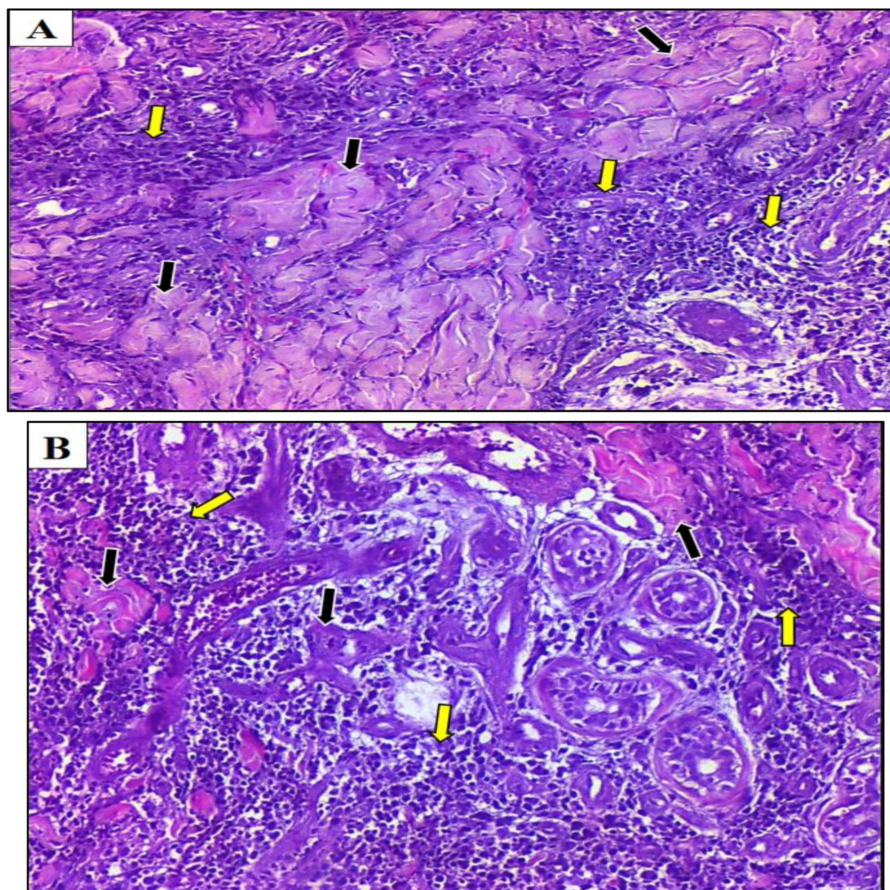


Figure (11): Photomicrograph of leishmania infected skin .

A / Hyperplasia of squamous epithelial cells of epidermis layer led to thickening of epidermis layer (black arrow) of infected skin. Also, necrosis of epithelial cells formed spaces (red arrow) in epidermis that filled with inflammatory cells were observed. Infiltration of inflammatory cells (yellow arrow) that aggregated as nests in dermis layer. B/ Note infiltration of inflammatory cells (yellow arrow) that aggregated as clusters in dermis layer. H&E. A: 100x and B: 40x.



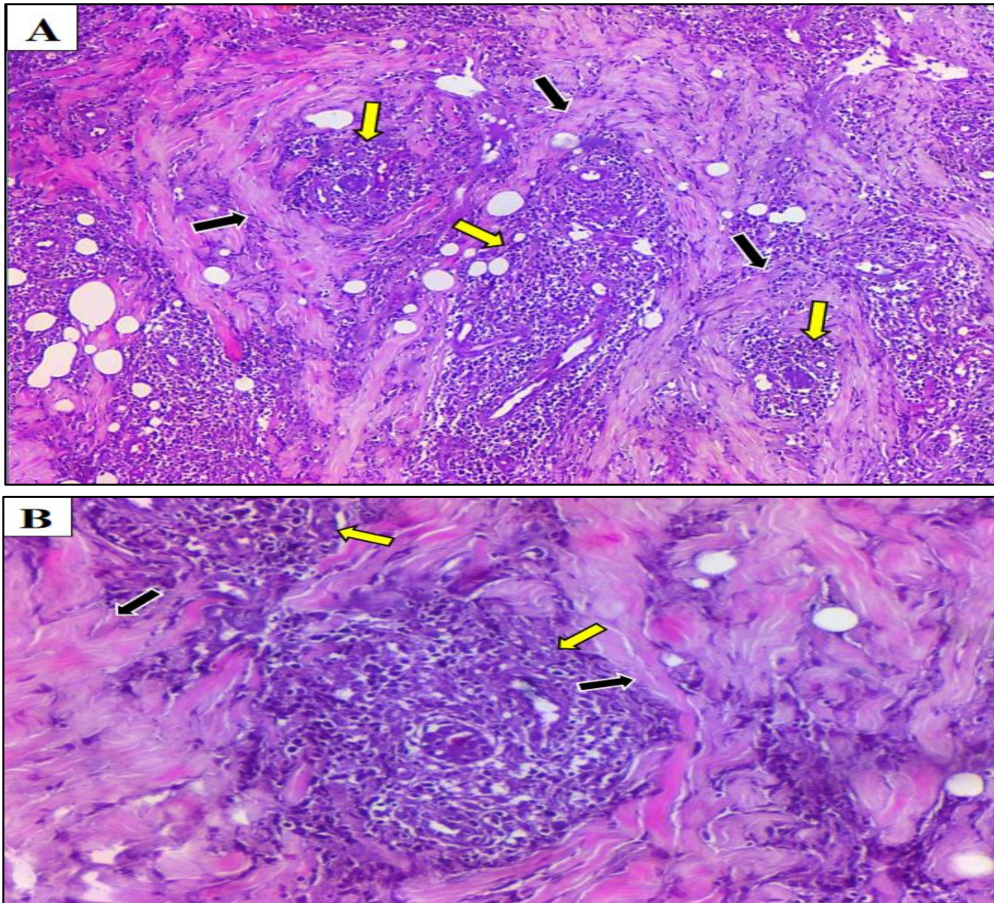
Figure(12): Photomicrograph of leishmania infected skin .

Journal of Chemical Health Risks

www.jchr.org

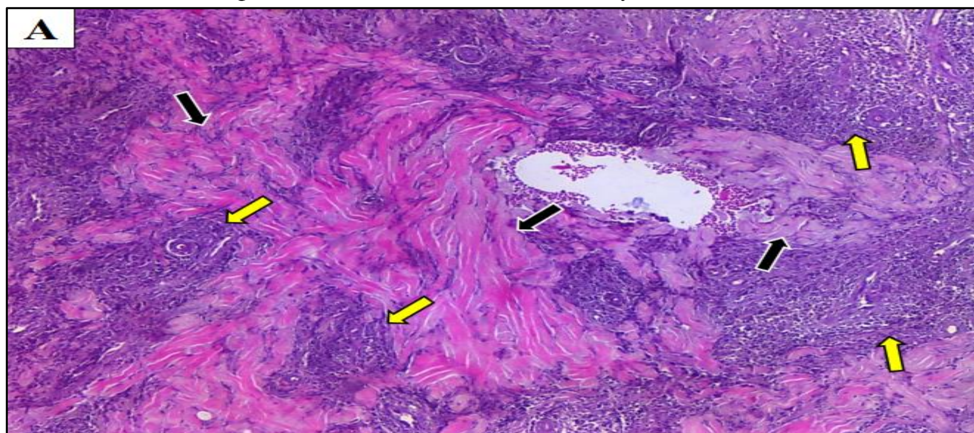
JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

A&B/ Massive infiltration of inflammatory cells (yellow arrow) mostly mononuclear cells with presence of fibrous tissue deposition (black arrow) in dermis layer of infected skin. H&E. A and B: 100x.



Figure(13): Photomicrograph of leishmania infected skin .

A&B/ Massive infiltration of inflammatory cells (yellow arrow) mostly mononuclear cells that surrounded by fibrous tissue capsule (black arrow) to form granuloma like features in dermis layer of infected skin. H&E. A: 40x and B: 100x.



Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

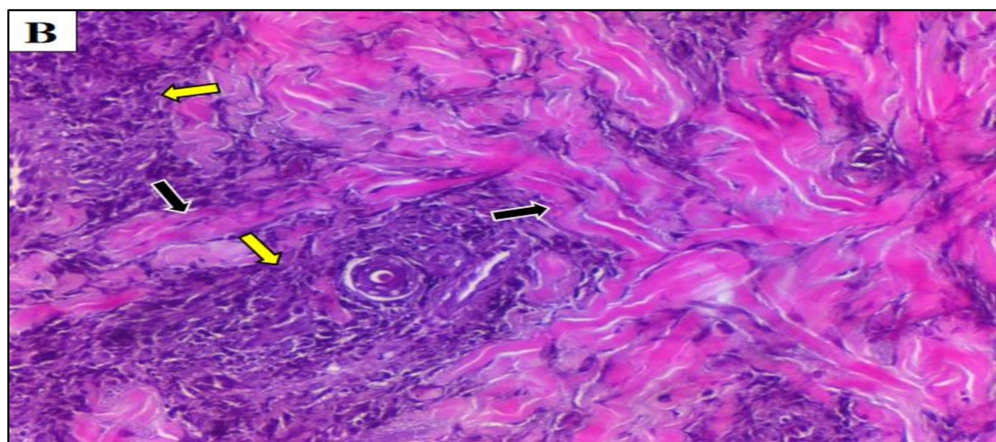


Figure (14): Photomicrograph of leishmania infected skin

A&B/ Massive infiltration of inflammatory cells (yellow arrow) mostly mononuclear cells that surrounded by fibrous tissue capsule (black arrow) to form granuloma like features in dermis layer of infected skin. H&E. A: 40x and B: 100x.

DISCUSSION

The diagnosis of CL is based on clinical features especially in endemic regions(Bari&Rahman.,2006) and laboratory testing. Many diagnostic methods have been described with different degrees in diagnostic accuracy, including direct parasitological examination (microscopy, histopathology, and parasite culture) and/or indirect testing with serology and molecular diagnostics (Goto & Lindoso,2010) .The histologic picture in CL varies according to the stage of infection and the clinical type. It is important to search for amastigotes, which are diagnostic (Bari& Rahman,2006; Venkataram *et al* .,2001; Mansour *et al.,* 1993; Rahman ,2003; Mashhood *et al*.,2005;(Koçarslan *et al*.,2013). Histopathological findings may be suggestive and occasionally diagnostics when the LD bodies are identified . Diagnosis of cutaneous leishmaniasis is mainly clinical by observing characteristic lesions especially in endemic areas, however definite diagnosis should be reached in cases of atypical lesions that might be confused with mycobacterial, mycotic or bacterial infections. Therefore definite diagnosis must depend on the isolation of causative organism by smear, culture ,and its identification in tissue section. (Schnur *et al* .,1987) . The confirmation of CL. Is done by demonstration of the amastigote parasite in sores (Burton,2009). This can be achieved by various smears; scalpel scrapping, by using a dental broach , fine needle aspiration , impression smear of skin biopsy(Fitzpatrick,1993; Qurban,2002)

Various studies were done to define the best technique to obtain the outmost results to identify the parasite and characteristic features in a smear or biopsy of the lesion. Our study results in histopathological biopsy with H&E stain (36.3%) were higher than the results of Mashood(2004) with 30.5% positive results for identifying cutaneous leishmania amastigote and Bhutto *et al*(2003) with 30.2% positive results and and Wiegele *etal*(1987) with just 13.9% positive results. Our results were lower to the results of Sultan *et all* (2009) with 59%, Rahman *et al* (2003) with 92% , Azadeh *et al* (1985) with 54.7% , Simeen *et al* (2003) with 76% and Sezen Koçarslan (2013) with 66.7%. Our study revealed 45.4% positive result for identifying cutaneous leishmaniasis amastigote using aspirate smear and geimsa stain, this result was close to, and higher than Nasser *et al,* (2005) with 32%, Rahman *et al* (2003) with 30% but lower to the results of Hepburn(2003) with 50-80%, Sultan *et all* (2009) with 66% .This variation in positive results could be attributed to skill of the investigator and technique used(Hepburn.,2003) . The success of demonstration of organism depends on a multitude of factors, including the site of biopsy and duration of lesion. Biopsy from floor or margin is best suitable for detection of parasites, though such a biopsy is difficult to obtain,(Tareen *et al.,* 2014) . Sharquie *et al* (2002) emphasized that multiple sites from the edge should be examined rather than one site because parasites are

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

present in a foci rather than distributed in the lesion. Parasites disappear from the biopsies after 5-7 months.(Cannizares .,1975). In late lesions less than 50% may show prasites.(Kurban *et al.,*1966). Occasionally parasites may not be detected even in earlier lesions. (Cannizares.,1975) This may be related to the early onset of epitheloid response . Organisms in the epidermis are reported to be rare(Grevelink .,1996). Kurban (1966). suggested that the presence of intraepidermal parasites was an example of trans epidermal elimination of the organisms. In our study we could identify the parasite in the epidermis in one slide (Figure3) this result is similar to Sezen Koçarslan *et al.,* (2013) who detected intra epidermal leishmania amastigotes in one case. A unique finding by Mohamed (2003): promastigote form was seen both by light and electron microscope in extracellular fluid.

Several classifications and patterns were suggested by authors, It is possible that different parts of a lesion and of the same section may show different stages of evolution and hence different histological patterns(Ridley.,1979). Shaquie *et al*(2002) classified lesion in early lesions dependent whether it is ulcerative lesion or not, in early lesions there were lymphocytic invasions of the dermis together with multiple foci of plasma cells , in the dry and in lesions with longer duration, the dermal changes were mainly granuloma formation with few lymphocytic cells and plasma cells. Epidermal hyperplasia with orthoparakeratosis was noticed in 18 patients, while in some cases the epidermis showed necrosis and atrophy. Still, some sections showed pseudo epitheliomatous hyperplasia. Follicular plugging and liquefaction degeneration of the basal layer was observed in 5 patients, lymphocytic exocytosis was also recorded in many patients. Mashood *et al*(2004) classification was in four pattern; Mixed inflammatory cell infiltrate with LT bodies and no granulomas, Mixed inflammatory cell infiltrate with LT bodies and presence of epithelioid cell granulomas ,Mixed inflammatory cell infiltrate, with no LT bodies and the presence of epithelioid cell granulomas, Mixed inflammatory cell infiltrate with no LT bodies and no granulomas. Mashood went along with Sharquie *et al*(2002) in his clinic correlation ; In wet and ulcerated lesions, the infiltrate was mainly mixed, and there were more chances of picking up of amastigotes in the histopathological slides. In dry and nodular lesions, the infiltrate was again mixed but there was a greater tendency of granulomas formation with less chances of picking up amastigotes. Kurban *et al(1966)* reported two histological patterns- early lesions (less than 1 year) showing a diffuse macrophage infiltrate, with plenty of organisms and late lesions (more than 1 year) with a granulomatous infiltrate. Mansour(1993) in his study reported similar pattern. Khalaf (2020) in a histopathological study in Iraq of CL. lesions skin biopsy revealed following results; marked epidermal hyperplasia with hyperkeratosis .two small granulomas of macrophages some with vaculation in the dermis sever dermal hyperplasia and foliculitis also hyperkeratosis and scab formation,marked hyperplasia of epidermis , hyperkeratosis and scab formation . In our study we identified early changes of the epidermis with various degrees of inflammatory cell infiltration , hyperplasia of epidermis and necrosis of epithelial cells and inflammatory cells infiltration in the dermis with a possibility to identify the amastigote without hyperkeratinization of squamous cells of epidermis and without fibrosis or granuloma in the dermis as shown in Figures (3, 4, 7, 8, 9, 11). The late lesion could be distinguished with hyper keratinization of epidermis , occasionally fibrosis on the epidermis , granuloma in the dermis and fibrosis of the dermis in Figures (2, 5, 6, 10, 12, 13). Amastigot appears as oval or round shape contains nucleus with anterior kinetoplast inside macrophage cells(WHO 2003; Arfan and Rahman ,2006; Gazozai ,2010) . The morphology of LD bodies (amastigotes)in smears was mainly spindle shape , other morphological forms such as barrel, round safety pin and umbrella-like were noticed in some smears together with spindle shape. In some smears the amastigotes were mainly seen extracellular but in a number of patients intracellular forms were also observed These LD bodies had the tendency to occur at the lymphocytic spectrum rather than in the granulomatous phase. The morphology of LD bodies in histopathological sections were rounded with a nucleus and kinetoplast, in some sections spindle shape form similar to smear morphology were detected. (Sharquie *et al.,*2002). Tareen *et al.,* (2014) described amastigote as LT bodies with kinetoplast and eccentric nucleus inside macrophage and extracellular scattered in dermis, amidst plasma cells and macrophages.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

In our study under 400x power of light microscope we could identify amastigote in necrotic cells spaces Figure(3) in the epidermis and within the inflammatory cells aggregation in dermis Figure(2). In early lesions the changes of diffuse macrophage infiltration, necrosis, plasma cells and presence of parasites is sufficient for diagnosis. Difficulties arise when organisms are scanty and there is a granulomatous infiltrate.(Mehregan *et al*.,1997; Kurban ., *et al* 1966 ; Paksoy&Hekin ,1993) In a skin biopsy showing histiocytic infiltration and/or a granulomatous reaction, CL should be included in the differential diagnosis.(Koçarslan *et al.,* 2013). A characteristic feature considered in his study(Sharquie *et al*., 2002) for the first time is finding Plasma cells in abundance in the early ulcerative wet lesions and were present in typical foci: So on any histopathological examination whenever we see plenty of plasma cells in sections, the examiner should check for cutaneous leishmaniasis. This goes well with a later study by Mashood *et al* (2004) : A constant finding in his study was the presence of a dense mixed inflammatory cells infiltrate in the superficial dermis. This led to a conclusion that this is not present in any other chronic granulomatous disease; hence, this should be taken as a diagnostic feature in the absence of LT bodies. We had similar results in slide (Figure13) which showed massive infiltration of inflammatory cells (yellow arrow) mostly mononuclear cells that surrounded by fibrous tissue capsule (black arrow) to form granuloma like features in dermis layer of infected skin and slide (Figure14) which showed Massive infiltration of inflammatory cells (yellow arrow) mostly mononuclear cells that surrounded by fibrous tissue capsule (black arrow) to form granuloma like features in dermis layer of infected skin. Venkataram *et al*.,(2001) Findings such as basal cell degeneration and intra epidermal abscesses, hyperplastic rete ridges though nonspecific, may be helpful in differentiating CL from other lesions.

CONCLUSION

The decisive finding in diagnosis of cutaneous leishmaniasis in various Laboratory histological methods is identifying amastigote in the slide. A massive infiltration of inflammatory cells in the superficial dermis is indicative to cutaneous leishmaniasis**.** Laboratory histological methods has a high specifity rate but with a low sensitivity rate in diagnosis of cutaneous leishmaniasis with high cost and require high experience and high technique to achieve acceptable results.

REFERENCES

1. Abadías-Granado I, Diago A, Cerro PA Palma-Ruiz AM, Gilaberte Y. Leishmaniasis cutánea y mucocutánea. ACTAS Dermo-Sifiliogr. 2021;112:601---618.).
2. Adini , I. R .L ; Jacobson , M., Kasap, Y., Schlein and C.L. Jaffe (1998) Species –specific detection of Leishmania in sand flies
3. Anderson K, Gaafar A, El-Hassan AM. Evaluation of PCR in the diagnosis of cutaneous leishmaniasis due to Leishmania major : acomparison with smears and sections from lesions. Trans R Soc Trop Med Hyg. 1996; 90: 133-35
4. Arfan B, Rahman ,S .Correlation of clinical, histopathological, and microbiological finding in 60 cases of cutaneous leishmaniasis. IJDVL .2006;72:28-32.).
5. Asher Ahmed Mashhood, Iqbal Khan, Shagufta Nazir Journal of Pakistan Association of Dermatologists 2004; 14: 210-214
6. Azadeh B, Samad A, Ardehali S. Histological spectrum of cutaneous leishmaniasis due to Leishmania tropica. Trans R Soc Trop Med Hyg 1985; 79: 631-6.
7. Bari AU, Rahman SB. Correlation of clinical, histopathological, and microbiological findings in 60 cases of cutaneous leishmaniasis. Indian J Dermatol Venereol Leprol 2006; 72: 28–32
8. Bhutto AM, Soomro RA, Nonaka S, Hashiguchi Y. Detection of new endemic areas of cutaneous leishmaniasis in Pakistan: a 6-year study. Int J Dermatol 2003; 42: 543-8.
9. Burton , Holden CA. Leishmaniasis . Rook s Text book of Dermatology 8th ed. Boston : Blackwell Scientific Publications;2009;326: 377-82
10. Burton, Holden CA. Leishmaniasis. Rook s test book of dermatology 8th ed..Boston: Blacwell Sciefintic Puplications: 2009: 1410- 20.
11. Cannizares O. Cutaneous leishmaniasis; in: Clinical Tropical Dermatology. Blackwell Scienti¦c Pubs. London. 1975;185-206.

12. Fitzpatrick T B, Dermatology in General Medicine Hardcover – March 1, 1993 Mcgraw-Hill Book Co; 4th edition 0070212074.2772-77
13. Gazozai SU, Iqbal J, Bukhari I, Bashir S. Comparison of diagnostic methods in cutaneous leishmaniasis (histopathology compared to skin smears).Pak J Pharm Sci 2010; 23(4):363-6
14. Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. Expert Rev Anti Infect Ther. 2010 Apr;8(4):419-33. doi: 10.1586/eri.10.19. PMID: 20377337.
15. Grayson W. Infectious Diseases of the Skin. In: Calonje E, Bren T, Lazar A, McKee PH, editors. McKee's Pathology of the Skin. 4 th ed. Philadelphia: Elsevier Saunders; 2012. p. 844-8.).
16. Grevelink SA, Lerner EA. Leishmaniasis. J Am Acad Dermatol 1996;34:257-272.[Google Scholar](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Grevelink%20SA,%20Lerner%20EA.%20Leishmaniasis.%20J%20Am%20Acad%20Dermatol%201996;34:257-272.)
17. Hepburn N C. Cutaneous leishmaniasis: an overview. J Postgrad Med [serial online] 2003DOI: 10.4103/0022-3859.928
18. Hussain, M. A., Dawod. K. M ., Khether, A. A. (2021). Gene Action, Heterosis and Combining Ability in Maize Hybrids B- Using Line x Tester Analysis. Kufa Journal for Agricultural Sciences, 13(2), 30–40. Retrieved from https://journal.uokufa.edu.iq/index.php/kjas/article/view/3653
19. Khalaf Amal Khudair (2020). Histopathological Changes Associated with Cutaneous Leishmaniasis in Patients with Baghdad Boil/South of Iraq. Medico-legal Update, January-March 2020, Vol.20, No. 1 DOI Number: 10.37506/v20/i1/2020/mlu/194410
20. Koçarslan S, Turan E, Ekinci T, Yesilova Y, Apari R. Clinical and histopathological characteristics of cutaneous Leishmaniasis in Sanliurfa City of Turkey including Syrian refugees . Indian J Pathol Microbiol 2013;56:211-5
21. Korzeniewski K.(2005) Health hazards in Iraq. Lekarz Wojskowy [Military Doctor]. 2005; 81:176-80
22. Kurban AK, Malak JA, Farah FS. Histological spectrum of cutaneous leishmaniasis. Arch Dermatol 1966;93:396-401.
23. Mansour L, el-Marhoumy SM,Eid MM, et al. Histopathological study of different clinical forms of cutaneous leishmaniasis. J Egypt Soc Parasitol 1993;23:591-597.
24. Mashhood AA, Khan IM, Nasir S, Agha H, Saleem M, Imran A. Fine needle aspiration cytology versus histopathology in the diagnosis of cutaneous leishmaniasis in Pakistan. J Coll Physicians Surg Pak 2005;15:71–3.
25. Mehregan DR, Mehregan DA, Mehregan AH. Histopathology of cutaneous leishmaniasis. Gulf J Dermatol Venereol 1997;4:1-9..
26. Mohamed Wael Tayseer La Revue de santé la Mediterranee orientale, vol. 15, N 5,(2009.)
27. NR Dar, Khurshid T. Comparison of skin smears and biopsy specimens for demonstration of Leishmania tropica bodies in cutaneous leishmaniasis. J Coll Physicians Surg Pak. 2005 Dec;15(12):765-7. PMID: 16398966.
28. Muh. Asriadi AM , Samsul Hadi , Edi Istiyono . (2023). Trend research mapping of differentiated instruction: A bibliometric analysis ., Journal of Pedagogical Research, 2023 - Volume 7 Issue 3, pp. 194-210 ., https://doi.org/10.33902/JPR.202320544
29. Avishikta Ray .,Satkar Prasad. 2023. Development and Assessment of a Polyherbal Formulation Containing Fractions of Aegle marmelos and Pedalium murex Leaves for its Anti-Diabetic Potential., *Latin American Journal of Pharmacy* ., Vol. 42., No. 6.
30. Arun Shanmugam, Karthik Raja Kumar, Cindy Jose. 2023. Assessment of Awareness about Diabetes Related Retinopathy among Type 2 Diabetes Patients- A Prospective Study. Latin American Journal of Pharmacy ., Vol. 42., No. 6.
31. Anju Singh., Satkar Prasad .2023. Phyto-Phospholipid Complexes: A Novel Method to Increase the Therapeutic Effectiveness and Bioavailability of Herbal Extract., Latin American Journal of Pharmacy ., Vol. 42., No. 6.
32. Ebru Aydın, Gülcan Mıhladız Turhan. (2023). Exploring primary school teachers' pedagogical content knowledge in science classes based on PCK ., Journal of Pedagogical Research, 2023 - Volume 7

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

Issue 3, pp. 70-99 ., https://doi.org/10.33902/JPR.202318964
33. Paksoy N, Hekin E. Comparative analysis of the ciinicopathological features in cutaneous leishmaniasis and lupus vu!garis inTurkey. Med Parasitol 1993;44:37-39.[Google Scholar]
34. Qurban Ali Khushk. Drought and disease stalk Dadu districts western parts . DAWN. 25th March,2002.
35. Rahman Sb, Bari Au. Laboratory profile in patients of cutaneous leishmaniasis from various regions of Pakistan. J Coll Physicians Surg Pak. 2003 Jun;13(6):313-6. PMID: 12814526.
36. Zeeshan, S. (2023): Importance of Environmental Education for Eradicating Environmental Issues. – Journal of Environmental Impact and Management Policy (JEIMP) 3(04): 1-5.
37. Gayathri G, et al. (2023). Studies on Morphological Parameters, Radius of Gyration, Correlation Length and Invariant of HPMC Polymer Doped With Ferrous(II) Oxide Using SAXS Method. Catalysis Research. 3(1):07.
38. Schalling HDFH, Oskam L (2002) Review: molecular biological applications in the diagnosis and control of leishmaniasis and parasite identification. Trop Med Int Health 7: 641-651.).
39. Schnur LF, Jacobson RL. Parasitological Techniques in the Leishmaniasis. Vol. 1. London (UK): Academic Press; 1987. p. 499.
40. Sharquie KE, Hassen AS, Hassen SA, Al-Hamamil A. Evaluation of diagnosis of cutaneous leishmaniasis by direct smear,culture and histopathology. SaudiMed J 2002: 23: 925-8
41. Simeen BR, Arfan UB.Laboratory profile in patients of cutaneous leishmaniasis from various regions of Pakistan. J Coll Physicians Surg Pak 2003; 13:313-6.
42. Shaikh Zeeshan. (2023). Importance of Environmental Education for Eradicating Environmental Issues. Journal of Environmental Impact and Management Policy(JEIMP)., ISSN:2799-113X, 3(04), 1–5. https://doi.org/10.55529/jeimp.34.1.5
43. Sultan BA, Al-Dhalimi MA. A comparative study of the diagnosis of Old World cutaneous leishmaniasis in Iraq by polymerase chain reaction and microbiologic and histopathologic methods. Int J Dermatol. 2009 Apr;48(4):404-8.
44. Eteng-Uket S, Iruloh BRN. Differential and interactional influence of socio-demographic variables on intellectual ability. Journal of Pedagogical Research. 2023;7(4):111-30. https://doi.org/10.33902/JPR.202320992
45. Tareen Attiya , Syed Afaq , Anwar ul Haque. Comparative Study of the Diagnosis of Cutaneous Leishmaniasis by Slit Skin Smear and Skin Biopsy for Histopathology. JRMC [Internet]. 2014 Jun. 30 [cited 2023 Jul. 17];18(1). Available from: https://www.journalrmc.com/index.php/JRMC/article/view/395
46. MEHMET METIN ÖZGÜVEN., MEHMET EMIN BİLGİLİ. (2023). THE EFFECT OF CLIMATE CHANGE ON AGRICULTURAL PRODUCTION., Quantum Journal of Engineering, Science and Technology, 4(3), 29-37
47. Venkataram M, Moosa M, Devi L. Histopathological spectrum in cutaneous leishmaniasis: A study in Oman. Indian J Dermatol Venereol Leprol 2001;67:294-298
48. Kavaz D. 2023. Characterization, Synthesis, and Evaluation of Antimicrobial, Anticancer Properties of Laurus nobilis-derived ZnO Nanoparticles. NanoWorld J 9(3): 55-61.
49. Supriya V, Poiba VR, Vangalapati M. 2023. Catalytic Activity of Nanocomposite: Synthesized Electrochemical Sludge using Iron Electrodes and Marine Algae (Fe3 O4 /marine algae NP) for the Degradation of Emerging Pollutant Malathion. NanoWorld J 9(2): 29-36.
50. Jessica Maria Soria Villanes, Mónica Evencia Poma Vivas, Camila Alejandra Traverso Castillo, Michelle Norma Antonio . (2023). El microbio, camino biológico a través de la especie humana., Boletín de Malariología y Salud Ambiental., Vol. 63, Núm. 2 (2023), p: 330-337.
51. Ruth Katherine Mendivel Geronimo, Jessica Coronel Capani, Oscar Lucio Ninamango Solis, Juan de Dios Aguilar Sánchez, Ana María Enriquez Chauca .(2023). Rasgos antropogénicos de los ciclos zoonóticos en el Perú ., Boletín de Malariología y Salud Ambiental.,Vol. 63, Núm. 2 (2023), p:338-349

JCHR (2023) 13(4s), 42-55 | ISSN:2251-6727

52. Rafaat, J. G. (2022). Effect of pit and plastic bag methods and some additives on chemical composition of grass-legume mixture silages. Kufa Journal for Agricultural Sciences, 14(1), 11–20. https://doi.org/10.36077/kjas/2022/140102
53. Weigle KA, de Davalos M,Heredia P et al. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. AmJ Trop Med Hyg 1987; 36: 489-96
54. WHO (2003) Communicable Disease Working group on Emergencies, HQ Division of communicable disease control, EMRO, WHO OFFICE, Baghdad. WHO Office, Baghdad. Communicable Disease Toolkit, Iraq crisis . WHO 2003:39-44.
55. WHO (2021).https://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html.status of endemicity of Cutanaeous Leishmaniasis:2021