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STUDY THE PREVALENCE AND HISTOPATHOLOGICAL CHANGES OF CUTANEOUS LEISHMANIASIS IN NASSIRRIYAH CITY \ THIQAR- PROVINCE

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Key words : Cutaneous leishmaniasis , Baghdad boil , histopathology.

ABSTRACT

Cutaneous leishmaniasis is an endemic disease in Iraq and it is become epidemic in Nassirriyah city recently . the current study has reported high rate of infection among population at any age and gender , location or occupation . males have recorded high infection compared with female and also there is some differences among age of patients with Baghdad boil .

The following histopathological study were achieved in cancer research unit for college of Medicine at University of Thiqr, targeting Nassirriyah city in south of Iraq where Baghdad boil is highly distributed during the period of during the period from August 2015 to April 2016. several of tissue (skin) biopsies were obtained by dermatologist under sterilizing condition and at Al-Hussain teaching hospital where the patients have entered for treatment . the result explain the following changing :

- (1) Granuloma like associated with several layers of vaculated cells filled with parasite around it
- (2) Dermal areas of chronic granulomatous inflammation mainly formed by vaculated macrophages filled with parasite .
- (3) Epidermis with keratinization and vaculated prickles cells undered it vaculated macrophages with parasite.

- (4) Small granuloma and area of granulomatous inflammatory reaction mostly formed by vacuolated macrophages with parasites , few lymphocytes present between the macrophages.
- (5) Nest like of vacuolated macrophages associated with dermal infiltration of lymphocytes .

INTRODUCTION

Leishmaniasis is a parasitic disease caused by haemoflagellate *Leishmania*. The disease is widespread and may cause serious health problems in communities throughout the Mediterranean regions and the Middle East, including Iraq [1-3]. There are an estimated 12 million cases worldwide, and there are about 1.5 million new cases of cutaneous leishmaniasis each year, of which over 90% occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru [4]. Old World disease primarily is caused by *Leishmania tropica* in urban areas and *Leishmania major* in dry desert areas [3].

In Iraq, two species are present: *L. tropica*, the agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, the agent of zoonotic cutaneous leishmaniasis (ZCL). Both ACL and ZCL were reported as causative agents of leishmaniasis in Iraq, but ACL is found mainly in suburban areas [5]. The disease is epidemiologically unstable, with large and unpredictable fluctuations in the number of cases. The total incidence rate of cutaneous leishmaniasis in Iraq varies from 2.3 / 100000 to 45.5 / 100000 [5].

Cutaneous leishmaniasis is characterized by one or more cutaneous lesions on areas where sandflies have fed. 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).(6)

The aim of the following study was to estimate the prevalence of cutaneous leishmaniasis and to explain the histopathological changes from it.

MATERIALS AND METHODS

- **Period of study** : the current study were started from distribution of the infection in the city at August from 2015 where collected about 3400 of cases during August from 2015 to April persisting to January and February from 2016.
- **Data collection** : Baghdad boil were clinically diagnosed by dermatologist in hospital and also by making direct skin smear to examine the presence of parasite in skin. Data were collected from all patients who entered to Al- Hussain teaching hospital in Nassiriyah city during the period from August 2015 to April 2016 and include : age , gender and location of patients , type and location of ulcer , number of ulcer .
- **Skin biopsy** : Biopsies were taken from patients by the same dermatologist and placed in 10% of formalin to prepare it for tissue processing (9) .
- **Histology** : Tissues biopsy were processed according (9), briefly the tissue from the skin collected and placed in 10% formalin for histopathological studies and dehydrated by several dilutions of ethanol alcohol dealcoholization with xylol, then embedded with paraffin wax blocked (3-5) mm thickness sections were obtained by microtome. The sections were put on glass slides deparaffinised with xylol, rehydrated by alcohol and stained by hematoxyline and eosin.

Statistical analysis :

Results were analyze based on Chi- square test using SPSS program .

RESULTS

- prevalence of infection :

A total of 3400 patient who entered Al- Hussain teaching hospital in Nassiriyah city suffering from Baghdad boil during the period started from August 2015 to April 2016 and they having the following data documented in tables and explained in the following pictures,



Fig(1) : explain the presence of Baghdad boil or cutaneous leishmaniasis

According to the age of patients , high rate of infection were reported among 10- 20 1800 (52.94%) years old of patients followed by 900 (26.47%) for patients with 1-10 years old infected with Baghdad boil .(Table ,1).

Table (1) : distribution of Baghdad boil based on the age of patients

No.	Age of patient (years)	Number of patient (%)
1-	1-10	900 (26.47)
2-	10- 20	1800 (52.94)
3-	20-30	300 (8.82)
4-	30- 40	104 (3)
5-	40- 50	30 (0.8)
6-	50-60	56 (1.6)

Significant differences $P < 0.01$

Males were reported 2810 (82.64 %) case of infection comparison with females 580 (17.05%) , as illustrated in table below :

Table (2) : distribution of Baghdad boil based on the gender of patients

No.	Gender of patient	Number of patients(%)
1-	Male	2810 (82.64)
2-	Female	580 (17.05)

Significant differences P< 0.01

The number of bites were also checked , since 3120 (91.7%) of patients were report infection with single bite in comparison with 280 (8.23%) for that with multiple bites:

Table (3) : Number of bites of insect on the body of patients

No.	Type of bites	Number of patients(%)
1-	Single bite	3120 (91.7)
2-	Multiple bites	280 (8.23)

Significant differences P< 0.01

The ulcer were distributed on the body of patients as : hand 1800 (52.94%), face 1000 (29.41%), legs 400 (11.76%) , feet 170 (2.9%) and neck 30 (0.88%) as illustrated in the following table :

Table (4): location of ulcer on the body of patients

No.	Part of body	Number of patients (%)
1-	Face	1000 (29.41)
2-	neck	30 (0.88)
3-	arms	1800 (52.94)
4-	thorax	0
5-	Abdomen	0
6-	Legs	400 (11.76)
-7	Feet	170 (2.9)

Significant differences P< 0.01

Dry ulcer is the type of ulcer that was highly distributed among patients 3400 (100%) with Baghdad boil in comparison with wet ulcer which is consist the total infection in the city , as tabled (5) below :

Table (5): type of ulcer on the body of patients

No.	Type of ulcer	Type of ulcers(%)
1-	Dry ulcer	3400 (100)
2-	Wet ulcer	0

Significant differences $P < 0.01$

- **Histopathological study :**

The following histopathological changing has associated with Baghdad boil , the clinical infection with cutaneous leishmaniasis cased by the protozoan *Leishmania tropica* , as illustrated in the following figures;

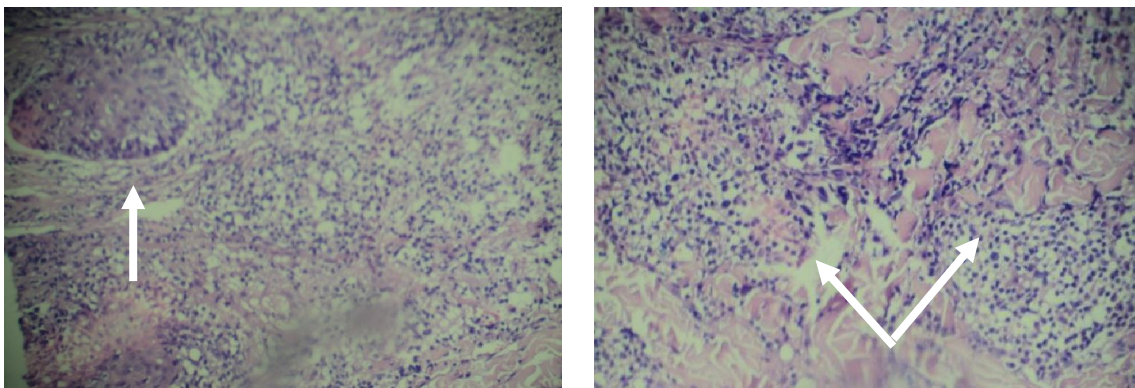
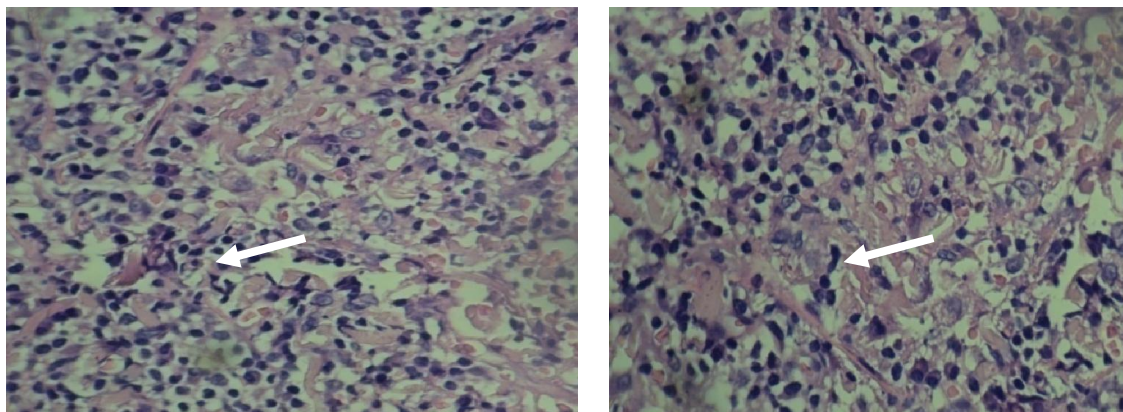
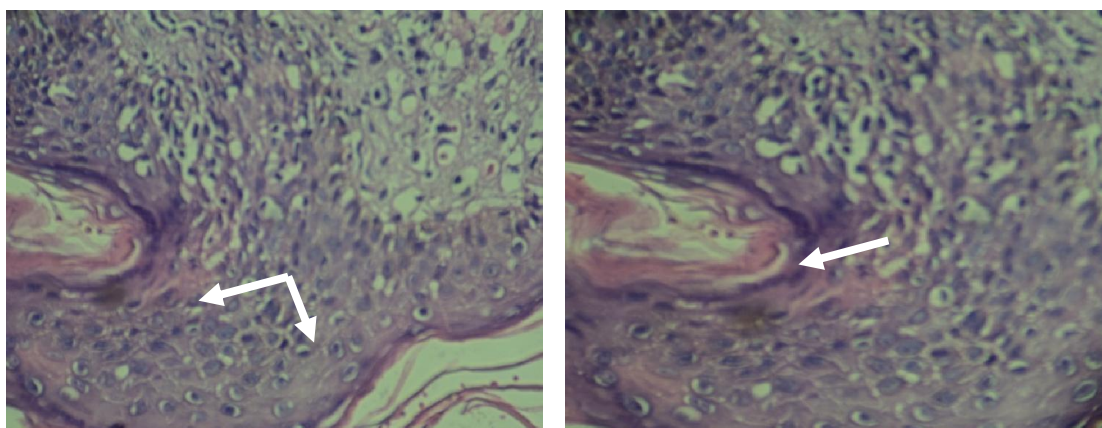


Fig (2) Granuloma like associated with several layers of vacuolated cells filled with parasite or LD bodies around it (10X , E&H).



Fig(3) :Dermal areas of chronic granulomatous inflammation mainly formed by vacuolated macrophages filled with parasites or LD bodies (40X, E&H)



Fig(4) Epidermis with keratinization and vacuolated prickly cells under it vacuolated macrophages with parasites (40X, , E&H)

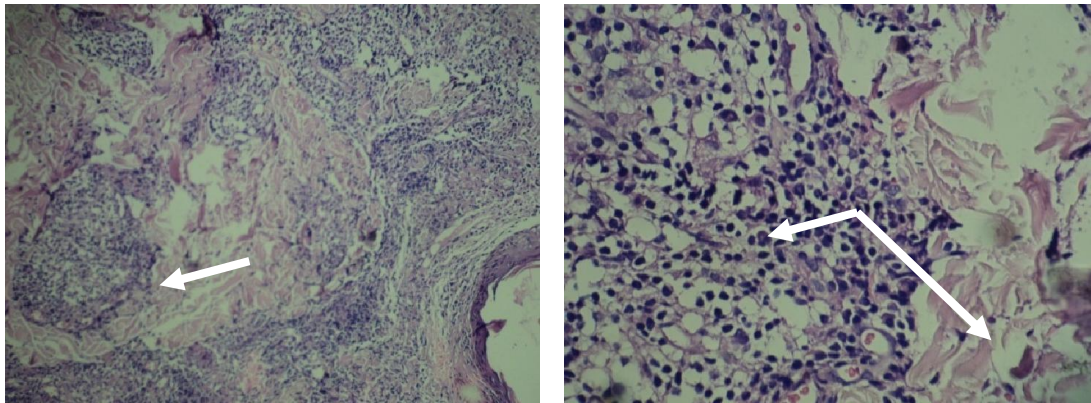


Fig (5) :Small granuloma and area of granulomatous inflammatory reaction mostly formed by vacuolated macrophages with parasites , few lymphocytes present between the macrophages (A 10X , B 40X , E&H)

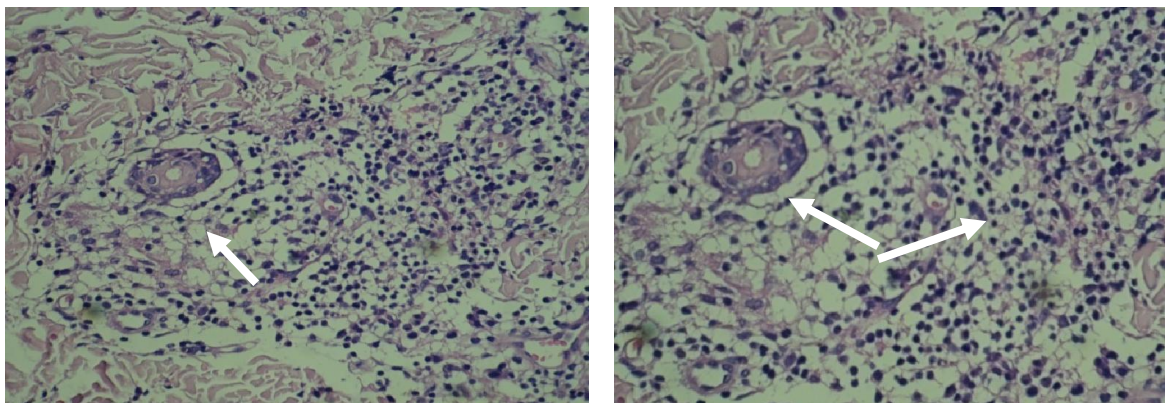


Fig (6) : Nest like of vacuolated macrophages associated with dermal infiltration of lymphocytes (40X , E&H).

DISCUSSION

There are many factors that play an important role in the presence and distribution of Baghdad boil in this district, including the presence of animal reservoirs such as rodents, dogs, . the presence of marshes; and the use of clay to build some of the houses in villages . Furthermore, Al- Nassiriyah district attracts and harbors many kinds of insects; therefore, its population are more exposed to insects bites (16) .

In this study the infection was reported about 3400 of cases during August to April persisting to January and February from 2016 . This rate was higher than those reported for other geographical areas in Iraq. In two community-based studies, the incidence was 2.5 case / 10,000 for Tikrit [11] in 2000 and 15 cases / 10,000 for Kirkuk city [12] in 2000. Additionally, in a hospital-based study performed in Samara [13], the incidence rate was 5.5 cases / 10,000 for the year 1994. However, the incidence rate was similar to that reported for Afghanistan [14] and Eastern Venezuela [15] .

Our findings also indicate that new cases of CL tended to increase in October and reach a maximum in January and February. The incidence rate of infection then starts to decline from March and reaches its lowest point in April. It was observed that the majority of CL patients attended the hospital between the months of October and March. This concurs with that reported by (16), but not with those reported for Iran [19] or Afghanistan [14]. This variation in seasonal peak could be due to the existence of various dominant reservoir species in each study area as well as to the activity of the sand flies. The differences in monthly distribution of CL patients might also be related to the development of female insects and their requirement of blood during their life cycle for the maturation and development of eggs, especially in spring season. The lapse of time between when the patient was bitten and the appearance of skin lesions might be related to the long incubation period of leishmaniasis (two to four months).

CL cases occurred more in males than in females. This result was in agreement with those reported by [16,17], [18 [19] for Iran, and Arfan for Pakistan. In contrast, [20] in Tikrit reported higher rates in females. These differences may be explained on the basis of variations between studies with regard to factors such as the size of the study population, the study design, climatic variations, and culture.

The incidence rate of CL in patients was higher in males as compared to females. This difference in the incidence rate of infection could be due to males in this age group playing outdoors without clothes and swimming in the rivers or lakes. This finding was in agreement with those reported for Iraq [16,21,]. The high incidence of ZCL may be due to the presence of reservoir animals in large numbers in this area, especially rodents and dogs. However, today, Afghanistan, Algeria, Brazil, Iran, Iraq, Peru, Saudi Arabia and Syria account for over of the 90% of the world's estimated 1.5 million cutaneous leishmaniasis cases [14,18]. Obviously, dense populations of natural ZCL hosts, together with abundant vector sand flies, are the key elements responsible for the high rate of human infection in the Nassiriyah area. ZCL in our

country may primarily affect farmers and nomads, who are chiefly exposed to night biting sand flies. In addition, the presence of high gerbil population densities in the area may be blamed as reservoirs of infection that are supported by the crops for which the irrigation canals had been constructed. Furthermore, the canal embankments serve as densely populated and favored rodent/sand fly infestation areas.

Histopathological changing of skin during cutaneous leishmaniasis explain the granulomatous reaction in the site of infection together with presence of amastigote stage or LD bodies in the lesion based on the current study. The essential feature of CL pathology is the colonization by amastigotes of cells of the mononuclear phagocytic system and the resulting granulomatous inflammatory response. A granuloma is defined as a compact collection of mature mononuclear phagocytes, not necessarily accompanied by accessory features such as necrosis. Conceptually granuloma evolves in three stages: 1) the development of an infiltrate of young mononuclear phagocytes; 2) the maturation and aggregation of these cells into an unorganized granuloma; and 3) the potential maturation of these cells into an epithelioid or organized granuloma (7,8,9,10)

Cutaneous Leishmaniasis presents a spectrum of manifestations both clinically and histologically. Lesions can present as nodule, plaque or ulcer mostly present on exposed sites (8,9). Histopathological findings in acute CL include dermal infiltrate predominantly consisting of macrophages containing large number of leishmania organism called LD bodies.5,6 In addition plasma cells and dense mixed inflammatory cell infiltrate are also present in dermis. When ulceration occurs secondary infiltration with neutrophils occur. (7, 8) 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.

دراسة التغيرات المرضية النسجية وانتشار داء اللشمانيا الجلدية في مدينة الناصرية / محافظة ذي

قار

امل خضير خلف ، صالح مجيد كاظم ، علاء عبد الحسن نايف

الخلاصة

يعتبر مرض اللشمانيا الجلدية من الامراض المتوطنه في العراق والتي انتشرت مؤخرا بشكل وباء في مدينة الناصرية / مركز محافظة ذي قار خلال الفترة بين شهر اب 2015 لغاية شهر نيسان 2016 . سجلت الدراسة الحالية معدل عالي لهذا المرض بين الاشخاص من كافة الاعمار ولكلا الجنسين ، اذ سجلت اعلى نسبة اصابة بين الذكور

اضافة الى بعض الاختلافات من حيث العمر . كما شملت الدراسة التعرف على التغييرات النسيجية الحاصلة في الجلد من خلال تحضير وفحص ودراسة المقاطع النسيجية في وحدة بحوث السرطان التابعة لكلية طب ذي قار بعد متابعة المرضى في مستشفى الحسين التعليمي التابع لمركز مدينة الناصرية ، وقد تم ملاحظة التغييرات التالية :

- 1- ظهور الطفيلي بشكل تجمعات ملتزمة داخل خلايا البلعم الكبير ذات مظهر حبيبي .
- 2- انتشار المظهر الحبيبي للطفيلي في مناطق متعددة من طبقة الادمه من الجلد والخاصة بوجود البثرة.
- 3- تصلب والتهاب مزمن في البشرة ناتج عن تجمع الخلايا البلعمية الحاوية على الطفيلي البيضوي الشكل .
- 4- بعض المناطق الحبيبية الحاوية على الخلايا البلعمية الملتزمة للطفيلي شهدت تجمع للخلايا الدموية البيضاء المفاوية.
- 5- كما ظهرت مجاميع لهذه الخلايا مع الخلايا الدموية البيضاء المفاوية في مناطق اخرى من البشرة.

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