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Antinuclear Antibodies Elevated in Cutaneous Leishmaniasis Patients: Diagnostic Implications

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Abstract. **General Background:** Leishmaniasis is a widespread parasitic disease transmitted by sandfly bites, posing significant public health challenges in developing countries, with cutaneous leishmaniasis being particularly prevalent in Iraq. **Specific Background:** While clinical diagnosis remains standard, immunological markers including specific antibodies (IgM, IgG) have been explored for cutaneous leishmaniasis detection, yet the diagnostic potential of antinuclear antibodies (ANA) remains underutilized. **Knowledge Gap:** The role of ANA as an immunological biomarker for cutaneous leishmaniasis diagnosis in Tikrit has not been comprehensively investigated. **Aims:** This serological study examined ANA levels and specific antibody responses in 60 clinically diagnosed cutaneous leishmaniasis patients at Tikrit Teaching Hospital from October 2023 to January 2024. **Results:** Males showed higher infection rates (53.33%) than females (46.66%); the 1-5 year age group exhibited the highest prevalence (31.66%); dry ulcers predominated (78.33%, $p<0.01$); 75% of patients demonstrated IgM antibodies, 5% showed IgG, and 20% tested negative; significantly, ANA levels were elevated in both male and female patients compared to controls ($p<0.05$). **Novelty:** This study establishes ANA as a valuable supplementary immunological marker for cutaneous leishmaniasis diagnosis in the Tikrit region. **Implications:** The findings suggest that ANA testing could enhance diagnostic accuracy and understanding of autoimmune responses in cutaneous leishmaniasis, potentially improving patient management strategies.

Keywords: Cutaneous Leishmaniasis, Antinuclear Antibodies, Immunological Markers, Serological Diagnosis, Tikrit Epidemiology

Highlights:

1. Dry ulcers predominated at 78.33%, significantly higher than wet ulcers at 21.66%.
2. Rural residents showed 71.66% infection rate compared to 43.33% in urban areas.
3. Seventy-five percent of patients exhibited IgM antibodies indicating acute infection phase.

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Introduction

Leishmaniasis is a widespread parasitic disease that poses a significant health problem for humans, particularly in developing countries, according to reports from the World Health Organization (WHO)(1). Many mammals, including humans, can be infected with these parasites, which are transmitted through the bite of the sand fly (2). It is a common disease in Baghdad, where it is locally known as "Baghdad boil", especially among children aged two to four years(3). It affects young adults and adolescents more than other age groups, with males being twice as likely to be affected as females. In China and Sudan, children under the age of five are significantly affected. An increase in leishmaniasis cases has also been observed in individuals with anemia, and malnutrition plays a major role in increasing susceptibility. Furthermore, the disease is increasing due to environmental changes and the ongoing large-scale migration from rural to urban areas(4). The disease is characterised by the formation of skin lesions that tend to heal spontaneously. The infection develops over a period of 2-3 months, with an incubation period of 1-4 months, during which ulceration may occur(5). The *Leishmania* parasite undergoes two stages: the first, the non-flagellated stage (a mastigote), parasitises the macrophages of the vertebrate host, and the second, the promastigote, parasitises the invertebrate host, the female sand fly (*Phlebotomus).* These two environments have specific and distinct physiological conditions, including temperature, pH, and osmolarity(6). Cutaneous leishmaniasis is diagnosed through clinical examination, but other diagnostic methods exist. Several methods can detect the *Leishmania* parasite, including immunological tests using swabs from clinically diagnosed samples, histological examinations, and the antinuclear ANA (antinuclear antibody)(7).

Methods

Collect of specimen:

Sixty cases of cutaneous leishmaniasis were diagnosed at Tikrit Teaching Hospital between October 2023 and January 2024. Information for each patient was recorded using a patient information form (questionnaire checklist). The diagnosis was made by a dermatologist at Tikrit Teaching Hospital.

Patient information:		
Form number:	Date the sample was taken: day-month-year	
Patient's full name:	Age:	Sex:
Accommodation	Region: District: City: Governorate:	
Site of infection	1-Head 2- Face 3- Upper limbs 4- Down limbs	
Type of infection	1-Dry 2-Wet	
Is there last infection	1-Yes 2-No	

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Another infection the house	1-Yes 2-No
Companion animals in the home:	1- Dogs 2- Cats 3- Rodents 4- Livestock 5- None

Serological test:

Serological tests were completed on 60 cases of cutaneous leishmaniasis at Tikrit Teaching Hospital, where blood (5 ml) was drawn from the infected and placed in sterile tubes (gel tubes) without anticoagulant. The samples were left at room temperature for 20 minutes, then transferred to a centrifuge to centrifuge at a speed of 5000 rpm for 5 minutes. After that, the serum was drawn using a micropipette and distributed into tubes. The serum samples were used directly for the purpose of immunological tests and detection of non-specific antibodies and specific antibodies belonging to the IgM and IgG classes, and the ANA test was performed.

Detection of specific antibody:

A quantity of serum was taken using a fine pipette of 80 microlitres and placed in the designated place for placing the sample in the test strip designated for testing for specific IgM and IgG antibodies. Then two drops of diluent solution (Figure 1) were added to it, and then it was left for 20 minutes until the result appeared on the test strip. When the indicator (red) appears on the control line (C), this indicates that the sample is negative. When the indicator appears on (G), this indicates that the sample is positive for specific IgG antibodies. When the indicator appears on (M), this indicates that the sample is positive for specific IgM antibodies.

Two drops of diluent **microliters of serum80**

Figure (1): Method for detecting the presence of specific antibodies

ANA detection:

Based on the leaflet included with the kit:

1-Remove the test device from the pouch and place on a clean and level surface.

2-Add 2 drops of Wash Buffer into the test window, waiting for the liquid to wet the membrane.

3-Add 50 µl of serum into the test window, waiting for the liquid to be absorbed completely.

4-Add 3 drops of Colloidal Gold Conjugate into the test window, waiting for the liquid to be absorbed completely.

5-Add 3 drops of Wash Buffer into the test window, and interpret the result within 3

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minute immediately after the liquid is absorbed adequa.

Statistical analysis:

The results were statistically analysed using the Analysis of Variance (ANOVA) program with the Chi-Square test at significance levels of (P≤0.0) and (P≤0.01) for individuals with cutaneous leishmaniasis and compared with the control group.

Result and Discussion:

A. Study by sex and age:

The current study did not show any significant differences in the incidence of cutaneous leishmaniasis based on gender, as shown in Table (1). The disease affects both men and women, and the higher incidence in males 53.33% compared to females 46.66% may be attributed to males' increased activity levels and the places they frequent outside the home, while females prefer to stay indoors. Consequently, males are more susceptible to bites from female sandflies. The study's findings are consistent with those of(8) in Salah al-Din Governorate, who confirmed a higher infection rate in males than females without any significant differences. The results also align with the 2018 study by(9) in Kirkuk Governorate, which found no significant difference in parasite prevalence between males and females. Furthermore, they are consistent with the findings of (10), whose infection rate was 50.8% in males and 49.2% in females. The study also presented the infection rate by age group, and no significant differences were observed. The fact that the disease affects all ages may be due to the fact that all age groups are exposed to sand fly bites, but the difference is due to the immune response, which varies from person to person. For example, children have a weak immune response, so they are susceptible to infection, in addition to their inability to repel insects. The results of the current study are consistent with(11), where the infection rate in children was 21.8%, higher than in adults by 1.8%, and are inconsistent with(12) in Diyala and with(13), where the infection rate in females was higher than in males.

Table (1): Rate of cutaneous leishmaniasis infection by age and Sex:

Age	male	Percentage%	Female	Percentage%	المجموع	Percentage%
Year-5	10	%31.25	9	%32.14	19	%31.66
10-6	6	%18.75	5	%17.85	11	%18.33
-11 20	4	%12.5	3	%10.71	7	%11.66
-21 25	6	%18.75	5	%17.85	11	%18.33
-26 30	2	%6.25	1	%3.57	3	%5
-31 35	-	-	-	-	-	**-**
-36 40	-	-	-	-	-	**-**

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-41 45	-	-	-	-	-	**-**
-46 50	2	%6.25	2	%7.14	4	%6.66
-51 55	1	%3.12	2	%7.14	3	%5
-56 60	1	%3.12	1	%3.57	2	%3.33
Total	32	%53.33	28	%46.66	60	% 100
Ns **Pearson Chi-Square = 0.781- P-Value = 0.819**						

B. Ns indicates no significant differences

2.1 Infection prevalence according to sex and month of study:

The current study showed a monthly distribution of cases among the months of the study in terms of the rate of infection with cutaneous leishmaniasis, where the highest infection rate was in December 45% and the lowest infection rate was in October at 11.66%, without significant differences, as shown in Table (2). This indicates that the spread of the disease is not related to a specific time of the year. This may be attributed to the continuity of the vector's activity and suitable environmental conditions throughout the year, or to the long incubation period, which leads to the overlap of the appearance of cases between different months, thus reducing the possibility of showing statistically significant differences.

The results of the current study are consistent with(14) in Salah Al-Din Governorate, where a monthly distribution of cases was found, but without significant differences between the months. They are consistent with what (15) concluded in their study on the epidemiology of cutaneous leishmaniasis, where the highest infection rate was in December at 35.59%. The results of the current study differ from what(16) recorded, where the highest infection rate was in April at 75% and the lowest infection rate was recorded in October at 55.6%.

Table (2): Infection prevalence according to gender and months of study

Study months	**Male**	**Percentage%**	**Female**	**Percentage%**	**Total**	**Percentage%**
October 2023	4	%12.5	3	%10.71	7	%11.66
November 2023	8	%25	7	%25	15	%25
December 2024	14	%43.75	13	%46.42	27	%45

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January 2024	6	%18.75	5	%17.85	11	%18.3
Total	32	%53.33	28	%46.66	60	% 100
Ns **Pearson Chi-Square = 0.120 - P-Value = 0921**						

C. Ns indicates no significant differences

3.1 Spread of infection by residential area:

The current study revealed that the highest infection rate was in rural areas, reaching 71.66%, while the percentage of infected individuals residing in the city was 43.33%, with no statistically significant differences as shown in Table (3). The study's results are consistent with (17), who indicated that those infected with cutaneous leishmaniasis residing in rural areas have a higher infection rate than those residing in cities. This may be attributed to the fact that most districts and sub-districts suffer from deteriorating service and health conditions and the accumulation of waste, as well as the lifestyle and social customs in rural areas. People are accustomed to spending evenings outside the home during the summer, and residents of districts and sub-districts prefer to live in open houses with animals in or near their homes, which act as reservoir hosts for the parasite, especially dogs and rodents. In addition, the presence of ponds and swamps provides a suitable environment for the growth of the insect vector. The geographical spread of cutaneous leishmaniasis depends on the geographical distribution of the insect vector, which is more prevalent in rural and humid agricultural areas(18).

The study did not agree with(19), who indicated that the prevalence of cutaneous leishmaniasis in the city is higher than in the countryside, reaching 60.48% and 39.52% respectively. The reason for the similarity of the percentages between the countryside and the city, i.e. the lack of significant differences, may be due to the presence of modern technology in our time, which has led to the spread of awareness and the trend towards hospitals to take Pentostam treatment for cutaneous leishmaniasis, and this in turn reduces the rate of infection with the disease.

Table (3): Infection rate by place of residence

Area of residence	**Male**	**Percentage%**	**Female**	**Percentage%**	**Total**	**Percentage%**
the countryside	18	%56.25	16	%57.14	34	%71.66
City	14	%43.75	12	%42.85	26	%43.33
Total	32	%53.33	28	%46.66	60	% 100
Ns **Pearson Chi-Square = 0.054 P-Value = 0.979**						

D. Ns indicates no significant differences

4.1 Spread of infection according to the location of the infection in the body:

The study included the distribution of infection in different parts of the body, as it

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was found that different parts of the body were exposed to the parasite. Table (4) showed no significant differences, as the highest percentage of cutaneous leishmaniasis infection was in the upper limbs at 28.33%, followed by infection of the face with the upper limbs at 21.66%, and the lowest percentage of infections throughout the body included the upper and lower limbs with the face, which amounted to 5%. This can be explained by the fact that most infections were in the upper limbs and face, as the arms and face are the most exposed parts, in addition to the habits of some people in the infected areas, where some people prefer to sleep in the open, so the upper and lower limbs and face are exposed, and therefore more exposed to being bitten by the sand fly.

The results of the current study are consistent with those of (20),where the highest percentage of lesions was found in the upper extremities 52.62% compared to other sites of infection, followed by the legs 26.32%, the feet 15.75%, and the face 5.26%. These results also agree with those of (16), who found that the upper and lower extremities and the face were the most commonly affected areas of cutaneous leishmaniasis, while the trunk had the lowest incidence. Several other studies, such as those by (21) and (22), conducted in various Iraqi governorates, have also indicated the presence of multiple lesions in the extremities.

Figure (2): shows skin ulcers resulting from cutaneous leishmaniasis

Table (4): site of injury in the body by sex

Location	Male	Percentage%	Female	Percentage%	Total	Percentage%
Upper limbs	9	%28.12	8	%28.57	17	%28.33
Face	6	%18.75	7	%25	13	%21.66
Lower limbs	5	%15.62	4	%14.28	9	%15
Upper limbs + Face	7	%21.87	6	%21.42	13	%21.66

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Upper limbs + Lower limbs	3	%9.37	2	%7.14	5	%8.33
Upper limbs + Face+ Lower limbs	2	%6.25	1	%3.57	3	%5
Total	32	%53.33	28	%46.66	60	% 100
Ns **Pearson Chi-Square = 0.642 P-Value = 0.968**						

E. Ns indicates no significant differences

5.1 The spread of the infection depends on the type of ulcer in the body.

The results of the current study recorded significant differences at the probability level (0.01), where the highest percentage of dry ulcers was 78.33%, while the lowest percentage of wet ulcers was 21.66%, according to Table (5). The study agreed with (23) in Najaf, where the percentage of dry ulcers reached 76.47% and wet ulcers reached 11.76% .It also agreed with (24) in DhiQar Governorate, where the highest percentage of dry ulcers was 72.1% and the lowest percentage of wet ulcers was 14%. The reason for the spread of the dry type in rural areas may be due to factors including socioeconomic status and malnutrition. Dry environmental conditions and delays in treatment may cause dry ulcers to persist for a longer period. The reason for the spread of the infection on the face may also be explained by the fact that the dry form of the infection tends to appear on the face in particular, while the wet type often appears on the arms and legs almost equally (25).(26) He stated that people with multiple ulcers have a higher immune response than people with a single ulcer.

Table (5): Number and percentage of cutaneous leishmaniasis cases according to the type of ulcer on the body

Type of ulcer	Number of injured	Percentage%
Dry	47	%78.33
Wet	13	%21.66
Total	60	% 100
** **Pearson Chi-Square = 38.777 P-Value = 0.0008**		

**** indicates that there are significant differences at the probability level (0.01)**

F. Detection of antibodies

The current study, which collected 60 blood samples from individuals infected with cutaneous leishmaniasis and performed serological tests, revealed no significant differences. The results showed that 75% of those infected exhibited IgM antibodies, 5% exhibited IgG antibodies, and 20% showed no antibodies, as shown in Table (6). These results are consistent with those of (27) in Baghdad, where no significant

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differences in antibody levels were found between infected and healthy individuals. This may be due to the fact that some patients have a strong immune response while others have a weak one. This variation results in a similar overall mean between the groups, leading to the absence of significant differences. However, the results of the current study do not agree with those of (28) ,who reported significant differences in antibodies between infected and healthy individuals. The detection of IgG antibodies to the parasite indicates infection, with IgG representing chronic infection, while the presence of IgM indicates more acute infections (29) and (30) explained that the presence of specific antibodies is an important diagnostic feature of the sera of those infected with cutaneous leishmaniasis, as these antibodies are among the most important immune defense mechanisms of the host against cutaneous leishmaniasis.

Table (6): Shows the detection of IgG and IgM antibodies

Sex	**IgM**	**Percentage%**	**IgG**	**Percentage%**	**Negative sample**	**Percentage%**	**Total**	**Percentage%**
Male	25	%71.42	3	%8.57	7	%20	35	%58.33
Female	20	%80	-	-	5	%41.66	20	%41.66
Total	45	%75	3	%5	12	%20	60	-
Ns								
Pearson Chi-Square = 2.286 P-Value = 0.319								

G. Ns indicates no significant differences

7.1 Detection of antinuclear antibodies (ANA) in patients with cutaneous leishmaniasis

The study results showed a significant increase in the level of antinuclear antibodies (ANA) in males and females infected with cutaneous leishmaniasis compared to the control group. The reason may be the secretion of large quantities of autoantigens as a result of tissue destruction caused by the parasite, which releases tissue antigens, and the process of autoantibody production. The study results are consistent with(31) and (28), whose study results showed an increase in the level of (ANA) in people infected with cutaneous leishmaniasis. They are also consistent with the study by (32), which showed an increase in antinuclear antibodies (ANA) in people infected with visceral leishmaniasis compared to the control group**.**

A link has been observed between cutaneous leishmaniasis and autoimmune diseases. The humoral response in individuals infected with cutaneous leishmaniasis is indicative of elevated levels of antibodies and antibodies specific to cutaneous leishmaniasis. Although some antibodies are unaffected by the infection, certain antibodies exhibit elevated levels in the serum of individuals infected with the *Leishmania* parasite. It has been observed that individuals with cutaneous leishmaniasis exhibit an increase in several autoantibodies, including antinuclear antibodies, against cells and humoral components infected with the parasite(33). Studies have also confirmed that cutaneous leishmaniasis can lead to the production of large numbers of IgM, IgG, and

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IgA antibodies after the activation of B lymphocytes to produce polyclonal antibody B, generating antibodies specific to Leishmania cutaneous leishmaniasis. This is in addition to the production of autoantibodies, such as ANA and other antibodies that stimulate *Leishmania* infection, including the activation of both types of antibodies. B and T lymphocytes, where the pathogenesis of cutaneous leishmaniasis showed a strong association with the formation and activation of cytotoxic T cells by activating them through another pathway via the formation of T-helper-1 cells and some other cytokines in response to infection with the *Leishmania tropica* parasite that causes cutaneous leishmaniasis (34).

Table (7): The mean ± standard error of antinuclear antibody concentration in patients with cutaneous leishmaniasis compared to the control group

Groups	**Numbers**	**(mIu/ml)ANA Mean ± SD**
Control	30	0.0188 ± 0.0141
Patients	30	* 0.219 ± 4.450

***Significant at (P <0.05)**

Conclusions

The highest incidence of cutaneous leishmaniasis was observed in males, higher than in females. The highest incidence was recorded for people aged 1 to 5 years. The highest incidence of the parasite was in December and the lowest in October. The highest incidence was observed in the upper extremities and face. The highest incidence was recorded for dry ulcers and a lower incidence for wet ulcers. It is widespread among rural populations. High levels of antibodies were also observed in people infected with cutaneous leishmaniasis compared to the control group, indicating a high immune response in infected people.

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