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THE USE OF ANA ANTIBODY IN THE DIAGNOSIS OF *LEISHMANIA*

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ABSTRACT : The current study was conducted during the period from the beginning of October 2018 to January 2019 for the diagnosis of leishmaniosis skin in the city of Samarra. A total of 55 clinical cases ofdermal leishmaniosis were examined from the patients at Samarra Hospital. With 52.7% reported in males and 47.2% in females of the total number of clinically infected samples. In the current study, the percentage of dermal leishmaniosis was determined according to the various epidemiological criteria (age of the host, site of infection, months of study, geographical location, occupation, and presence of vector carriers and host in the host environment). The highest percentage of total infection was between 45 and 50 years of age (1.8%). The highest rate was recorded in December of 38.1. The highest infection rate according to the site of infection was 49% in the outskirts of the city, 70.9% in the city and 29% in the countryside. The results showed that 74.5% of the patients showed IgM antibodies, 5.4% showed IgG antibodies and 20% did not show any antibodies and the infection was diagnosed by the antibody examination and found an increase in the level of both males and females compared to control group.

Key words : Leishmania, antibodies, diagnosis.

INTRODUCTION

Leishmaniasis is the result of various types of *leishmania* parasites. The species of *leishmania* is one of theProtozoa, obligate and flagellate prasite. The *leishmania* parasite infects the immune system cells of the final host (human) by means of the sand fly, which represents the parasite carrier. This parasite is spread in the host body, depending on its type. The infection is either a skin that causes deformities and skin ulcers ,the infection is then known as cutaneous leishmaniasis. This parasite may infect the internal organs (such as the liver and spleen) of the host, causing hereditary injuries is then called "Visceral leishmaniasis" (Getti *et al*, 2009).

Leishmaniasis is a health problem caused by one of the common types of *leishmania,* called *Leishmania tropica*. The habitat of this species is in most tropical and subtropical countries, and dermatitis is often associated with low living standards and poor economic conditions of countries. Leishmaniasis affects about 12 million people in 88 countries and between 2 and 3 million new cases diagnosed each year. And 350 million people worldwide are at risk of developing leishmaniasis (WHO, 2014). Iraq is a major contributor to leishmaniasis, so it is commonly called Baghdad boil. As well as many other names (Oriental sore, Aleppo boil and Delhi sore) depending on the geographical areas spread by the disease.

Two of these parasites have been identified in Iraq: *Leishmania tropica* and *Leishmania major* (Mina *et al*, 2010). These two species are similar in life cycle. However, the clinical manifestations of the disease resulting from each infection are different. In terms of host and transporter and the response of the host and reactions to the direction of each type is also different is the incidence of leishmaniasis skin with the presence of one or more pimples of varying sizes when they appear, and the blister painful in the case of injury Balchmania the ancient world, and painful in the injury Balchmania modern world . The infection is associated with swollen glands and lymph nodes near the blister. Blister contains live and dead cells with dead parasite residues (CDC, 2012).

It also contains large infected and non-infected parasite cells with lymphocytes and plasmid constituents of Granuloma with heavy infiltration of neutrophil, acidophilus and macrophage cells. Cardiovascular leishmaniasis is diagnosed by clinical diagnosis, but this does not prevent other diagnostic methods. There are many methodes to detect leishmaniasis parasites, including histological and immunological tests from clinically diagnosed specimens and the antinuclear antibody ANA (Fakhar *et al*, 2012).

The studies in Iraq on the epidemiology of cutaneous leishmaniasis and its spread is inadequate and disproportionate to the seriousness of this disease, especially after taking into account the increase in cases recorded in recent years and significantly. According to the lack of epidemiological studies related to the spread of this disease in Salah-addin Governorate, so the current study aim to:

1. Determining the cutaneous leishmaniasis in Samarra city for patients, who visit the Samarra hospital according to some epidemiological criteria (months of the year, age of the host, presence of injuries in the same family, previous injuries, geographical location, profession, type of accommodation and the presence of carrier and feeders in the host environment).
2. Detection of acute and chronic infections by antibodies IgG, IgM method of rapid test (Cassite)
3. Detection of ANA infection.

MATERIALS AND METHODS

Collect of the specimen

In the present study, 55 cases of dermatitis were diagnosed in Samarra Hospital. During the period from October 2018 to January 2019. The information for each patient with leishmaniasis was written according to the patient information form (Table 1). The injury was diagnosed by a dermatologist at Samarra General Hospital.

Serological tests

Serological tests were performed on 55 cases of leishmaniasis at the Samarra General Hospital where blood samples were placed in sterile tubes lack of anticoagulation. Samples were left at room temperature for about 45 minutes, then transferred to the centrifuge for centrifugation at 3000 cycles/min for 5 minutes. Then pull the serum mediated by micropipette, and distributed in pipette tubes. Serum samples were used directly for the purpose of immunological examination for the purpose of detection of non-specific antibodies, in addition to detection of specific IgM and IgG antibodies. And ANA test.

Detection of specific antibody

The amount of serum was withdrawn by a micropipette of 20 microliters and placed in the specimen placement area of the test strip to examine specific IgM or IgG antibodies. Then two drops of Diluent Solution were added to it (Fig. 1). Then leave for 15 minutes until the result appears in the examination strip. When the indicator (red) appears on (C) Control line, it indicates

Fig. 1 : Method of detecting the presence of specific antibodies (Leaflet).

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 IgM-specific antibodies.

Two drops of dilution 20 microliters of serum.

ANA detection

1. Pipette 100 ul of calibrators, control and prediluted patient samples into the wells
2. Incubate for 30 minutes at room temperature (20-28C)
3. Discard the contents of the microwells and wash 3 times with 300 ul of wash solution
4. Dispense 100 ul of enzyme conjugate into each well
5. Incubate for 15 minutes at room temperature
6. Discard the contents of the microwells and wash 3 time with 300 ul of wash solution
7. Dispense 100 ul of TMB substrate solution in to each well.
8. Incubate for 15 minutes at roomtemperature
9. Add 100 ul of stop solution to each well of the modules
10. incubate for 5 minutes at room temperature
11. Read the opical density at 450 nm and calculate the result, the developed colour is stable for at least 30 minutes. Read during this time (Castro and Gourley, 2010; Defendenti *et al*, 2011).

RESULTS AND DISCUSSION

Study according to sex and age

The results of the present study showed that the percentage of infection by age groups shown in Table 2 a difference in the percentage of dermal dermatitis according to the age of the infected patients, ranging from a few months to 50 years. The highest rate of total infection was among those aged between months and 5 years (21.8%) for all patients in Samarra General Hospital, while thelowest percentage of total infection was at 45-50 years old (1.8%). No injuries were reported between the ages of 26-40 years for both sexes. The study showed increased incidence of leishmaniasis in children compared with adults, due to the lack of awareness of the child during the stinging of the insect and the weakness of the immune system, this results corresponding with the sudy

Table 1 : Questionnaire form.

No.:————————— History of infection—————

Mane	Sex		Age	The history of infection	The address	The gob
	Male	Female				
Is there last infection	yes	no				
Another infectioin the house	yes	No				
Building type of patient house			block	clay	stone	others
Animals in the house	doges		Cats	rodents	cattle's	No more
Type of infection	Site of infection		Number	dimeter	Treated before	
1. Wet	1. Face 2. Upper limbs 3. Downlimbs				1.Yes 2. No	
2. Dry	1. Face 2. Upper limbs 3. Downlimbs				Yes No	
3.						

Table 2 : The incidence of cutaneous leishmaniasis by age and sex.

Age	Male	Percentage%	Female	Percentage%	Total	Percentage%
5	7	24.1	5	19.2	12	21.8
6-10	6	20.6	2	7.6	8	14.5
15-11	3	10.3	7	26.9	10	18.1
20-16	6	20.6	5	19.2	11	20
21-25	5	17.2	6	23	11	20
26-30	-	-	-	-	-	-
31-35	-	-	-	-	-	-
36-40	-	-	-	-	-	-
41-45	2	6.8	-	-	2	3.6
46-50	-	-	1	3.8	1	1.8
	29	52.7	26	47.2	55	

Statistical analysis indicates that there were significant differences by sex and the months of the study at ($p < 0.05$)

by Al-Rasheed (2013), AL-Deffai (2013), AL-Azawi (2015) and Al-Hassani (2016), while not consistent with the study of AL-Tuffali (2003) where observed. The highest rate of infection in adolescence and youth age.

The study also showed that the percentage of male infection (52.7%) is higher than the percentage of female infection (47.2%). This may be due to the fact that males are more compatible with the external environment and are more susceptible to insect bites and infection, as well as to social traditions where male body parts are more susceptible to insect bites being exposed than females. These results are consistent with Kadhum (2012) in Diyala and AL-Deffai (2013) in Dewania city and not convenientwith study of Musa (2011) where females were more affected than males.

Prevalence of infection according to sex and months of study

The study showed that the highest rate of infection was in December (38.1%) followed by November (30.9%) and the lowest percentage in October (12.7%) (Table 2). These results are suitable with study of AL-Nasseri (2009) In Salah aadin and AL-Dawaneg (2014) in Maysan and Jaafar *et al* (2014) in Karbala.

Prevalence of infection by location of injury in the body

The results indicated in Table 4 shows that the highest incidence was in the upper limbs by 49%, followed by the infection of the face with the upper limbs by 18.1% and the lowest proportion of injuries throughout the body, which include upper limbs with the lower with the face, which amounted to 3.6%. Most of the injuries in the upper limbs and the face were the arms and face which arethe most exposed parts of the vector and these results were compatible with Al-Shuker (2012), AL-Rasheed (2013), Al - Azzawi (2015) and Al - Hassani (2016) and not compatible with AL-Deffai (2013).

Table 3 : The incidence by sex and months of study.

Months of study	Male	Percentage%	Female	Percentage%	Total	Percentage%
October 2015	4	13.7	3	11.5	7	12.7
November 2015	8	27.5	9	34.6	17	30.9
December 2015	10	34.4	11	42.3	21	38.1
January 2015	7	24.1	3	42.3	10	18.1
Total	29*	52.7	26*	47.2	55	-

Statistical analysis indicates that there were significant differences by sex and the months of the study at ($p < 0.05$).

Table 4 : The location of the infection by sex.

Location	Male	Percentage%	Female	Percentage%	Total	Percentage%
Upper limbs	15	51.7	12	46.1%	27	49%
face	2	6.8	2	7.6 %	4	7.2 %
Lower limbs	5	17.2	4	15.3 %	9	16.3 %
Upper limbs+ face+ Lower limbs	1	3.4	1	3.8 %	2	3.6 %
Upper limbs+ face	4	13.7	6	23 %	10	18.1 %
Upper limbs+ Lower limbs	2	6.8	1	3.8 %	3	5.4 %
Total	29*	52.7	26*	47.2	55	

Statistical analysis indicates that there were significant differences by sex at ($p < 0.05$).

Table 5 : The percentage of infection by place of residence.

Area of residence	Male	Percentage%	Female	Percentage %	Total	Percentage%
City	23	79.3	16	61.5	39	70.9
The countryside	6	20.6	10	38.4	16	29
Total	29*	52.7	26*	47.3	55	-

Statistical analysis indicates that significant differences were found by area of residence at ($p < 0.05$).

Prevalence of infection by area of residence

The results of the geographical distribution showed that infection in the city was 70.9% and in the rural areas 29% as in Table 5. The increase in cases was observed in the city. This is confirmed by the history ofcutaneous leishmaniasis. In endemic areas, the increase in the number of infections and their emergence in urban areas may be due to population migration, urbanization towards agricultural areas, environmental changes, the use of courtyard houses, and the opening of doors and windows during summer sleep when power cuts this is favor good activity for the insect, this is corresponding with Daham and AL-Alossi (2011), Abu Dawainj (2014) and AL-Deffai (2013), while they did not agree with Kadum *et al* (2014) and Rahi (2013).

Detection of antibodies

The results of the study showed that 74.5% of the patients had IgM antibodies, 5.4% had IgG antibodies, and 20% did not show any antibodies as in Table 6. Ozbilge *et al* (2000) found that the presence of specific antibodies is an important diagnostic characteristic of the leishmaniasis patients. These antibodies are the most important defense mechanisms of the host against cutaneous leishmaniasis. The detection of antibodies specific to the IgG parasite is evidence of the presence of infection. Chronic incidence of IgM was the most severe infection (Roitt *et al*, 2001) (Table 7).

In the current study, we found that leishmaniasis caused an increase in ANA levels in both male and female this agree with a finding by Ozlem (2013) and Hussein (2017). This may be due to of auto-immunity in leishmanial infection due to release a large amount of auto-antibodies as a result of the destruction of parasite-induced tissues. The release of histologic antigens may release the autologous process and produce antibodies. In the current study, we noticed an increase in ANA. This is consistent with the Ciaramella (1997) study of dogs with cutaneous leishmaniasis and was also consistent with Evangelos (2013) were ANA elevated in people with visceral leishmaniasis.

Table 6 : The detection of antibodies IgG, IgM.

Sex	IgM	Percentage %	IgG	Percentage %	Negative sample	%	Total	%
Male	20	68.9	3	10.3	6	20.6	29	52.7
Female	21	80.7	-	-	5	19.2	26	47.2
Total	41*	74.5	3*	5.4	11*	20	55	-

Statistical analysis indicates significant differences according to antibodeis at($p < 0.05$).

Table 7 : Mean ± SD. concentration of ANA (mIu/ml) of patients compared to control group.

Group	Number	ANA Mean ±SD
Control	30	4.450323± 0.219203
Patients	30	0.0188± 0.001414214*

* Significant at ($P < 0.05$).

There is a link between the infection of leishmania and autoimmune diseases, where the humoral response of patients with leishmania is an indication of increased levels of immunoglobulin and the specialized antibody of leishmaniasis, although there are some of the globulins not affected by this infection, but there are large numbers of those immunoglobulin levels in the serum of people with leishmaniasis (Bary, 1976). In groups studied and diagnosed with *leishmania* parasites, an increase in the number of autoantibodies including Antinuclear Antibody ANA against cells and humoral (Carvalho *et al*, 1983; Pearson *et al*, 1983).

Studies have also shown that cutaneous leishmaniasis can lead to the production of large numbers of IgG-IgM-IGA antibodies after activation of immune B cells Lymphocytes to produce antibodies to polyclonal antibodies for B lymphocytes produced antibodies specialized for injury alongside the production of auto antibodies, including ANA and other antibodies and the immune response to the infection and the stimulant caused by *leishmania* parasites.Including the activation of both B and T lymphocyte, where parasitic infection showed a significant correlation with the formation and activation of T-cytotoxicity cells by activating them through another route through the formation of T-helper-1 cells and the first aid and some other cytokines in response to a *Lashmania* parasitic infection (Malla and Mahajan, 2006).

CONCLUSION

The highest rate of total infection was among those aged between months and 5 years. The percentage of male infection is higher than the percentage of female infection. The highest rate of infection was in December followed November and the lowest percentage in October. Mot infection found on the upper limbs and it is highly prevalent among city residents. In the current study, we found that leishmaniasis caused an increase in ANA levels in both male and female.

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