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Epidemiological and clinical study of leishmaniasis in Kirkuk city, Iraq

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Abstract
leishmaniasis is a disease of global concern, *Leishmania* types and distribution rates vary from country to another. For visceral leishmaniasis serum samples were examined by the kala-azar dipstick rapid test. Meanwhile smears were taken from lesions, air dried, fixed and Leishman stained then microscopically examined for amstigote stage in cutaneous leishmaniasis. From April 2014 to April 2015, 150 samples for cutaneous leishmaniasis (CL) and 108 serum sample for visceral leishmaniasis (VL) from in and out patients in Pediatric, Azadi Teaching and Kirkuk General Hospitals were examined. The incidence of visceral leishmaniasis was very low (2.7%) comparing to that in cutaneous leishmaniasis (64.6%). No significant differences had appeared between the cutaneous leishmaniasis incidence in male or female. The most age group infected with cutaneous leishmaniasis in both genders was 5 ≤ years old, with rate of 26.1% in male and a rate of 15.8% in female. Significantly high frequency of the patients were presented with only one or two lesions with rate of 39.17, 25.7 % for each type of lesions respectively. The most location that infected with CL was hand (27.7%) and high rate of them being moist type (71.13%). In Kirkuk city CL is more prevalent than VL. High rate of CL is moist type locating in the limbs and face. The infection is more frequent in children than in adult. For CL a smear taken from the lesion is much worthy than a lesion blood sample or re aspirated saline injected to the lesion.

Keywords: Leishmaniasis, Epidemiology, Kirkuk city.

دراسة وبائية سريرية لداء الليشمانيات في مدينة كركوك/العراق

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الخلاصة
داء اللشمانيات هو مرض ذو أهمية عالمية، أنواع اللشمانيات و نسب توزيعها يختلف من دولة الى اخرى. لداء أللشمانيا الأحشائي أخذ عينات مصل وفحص بالطريقة السريعة باستخدام كلازار dipstic ، للنوع الجلدي اخذ مسحات من الأفة وضع على شريحة زجاجية ثم جفف و ثبت و صبغ بصبغة لشمان لفحصها مجهريا للبحث عن الاطوار غير المسوطة. اجريت الدراسة للفترة من شهر نيسان ٢٠١٤ و لغاية نيسان من عام ٢٠١٥ .فحص ١٥٠ عينة للشمانيا الجلدي و ١٠٨ عينة مصل للشمانيا الأحشائي. جمعت العينات من مستشفيات أزادي التعليمي، كركوك العام و مستشفى الأطفال العام. سجل نسبة قليلة

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(٢.٧%) للنوع الأحشائي مقارنة بنسبة ٦٤.٦% للنوع الجلدي. لم يظهر فرق معنوي لانتشار الطفيلي بين الإناث و الذكور. المرحلة العمرية الأكثر إصابة بالنوع الجلدي من كلا الجنسين كان لعمر ٥ سنوات واقل ونسبة ٢٠.١% في الذكور، و نسبة ١٥.٨% في الإناث. نسبة عالية معنويا من المصابين كان لديهم قرحة واحدة او أثنين و بنسبة ٢٥.٧% و ٣٩.١٧% لكل نوع على التوالي. المكان الأكثر اصابة في الجسم للنوع الجلدي كان الايدي (٢٧.٧%). نسبة كبيرة من الآفات الجلدية كان من النوع الرطب. يستنتج من الدراسة بان اللشمانيا الجلدية في مدينة كركوك اكثر انتشارا من النوع الحشوي. نسبة عالية من اللشمانيا الجلدي هو من النوع الرطب ويقع على الاطراف والوجه. ينتشر الاصابة بنسبة اكبر في الاطفال منه في البالغين. لتشخيص النوع الجلدي اخذ مسحة من الافة يكون مجديا اكثر من عينة دم الافة او حقن محلول ملحي ثم اعادة سحبه لعمل مسحة.

Introduction

Leishmania belongs to Tryponosomatidae family, is an intracellular protozoa parasite responsible for leishmaniasis disease in human and animal [1]. The main mode of transmission is through a sand fly bite [2]. The parasite has two distinct stages, the promastigote (motile extracellular form) in the sand fly gut and the amastigote (none motile intracellular form) in human phagocytes and reticulo-endothelial systems [1, 2]. About 12 million people infected with *Leishmania* in 98 countries. *Leishmania* exists in three forms cutaneous, mucocutaneous and visceral leishmaniasis. Cutaneous leishmaniasis is the most dispersed form, leading to a dry or moist sore on the skin, which may heals after a while, leaving a scar [3]. Mucocutaneous leishmaniasis effects nose and mouth mucosal membranes and skin. Visceral leishmaniasis or Kala-azar is the furthermost serious form, producing hepato-splenomegaly and anemia, it may be fatal if left untreated [4]. A number of studies on CL and VL have been done in Iraq, in Alhaweja, Kirkuk, of 23,778 persons, 107 were positive for cutaneous leishmaniasis, with an incidence rate of 45 cases per 10,000 [5]. Different rates of CL were recorded using different diagnosing methods, direct microscopy 60%, culture media 85%, isoenzyme analysis 100% [6].90.4 % of leishmanin skin tested patients (198) were positive for CL [7]. Among 100 cases of cutaneous leishmaniasis, 23 were wet type and 77 were dry type [8]. A study had concluded that Middle Eastern provinces in Iraq are at greater risk for leishmaniasis because of the migration from other parts [9]. A rate of 97.8 % was positive for CL by direct Giemsa stain and lower percentage by different culture medias [10]. 85.4% and 81.25% of serum samples examined by ELISA and IFAT, respectively were positive for VL [11]. The aim of the present study was to find out the rate of cutaneous and visceral leishmaniasis in Kirkuk city because most of available studies don't referrers to the exact prevalence of each type of *Leishmania*, in addition to that it preferable that such studies done annually or at least from time to time to know the certainties of these diseases existence and their rates in our city.

Materials and methods

Samples collection**:** From April 2014 to April 2015, 150 sample for CL and 108 serum sample for VL were eligible for this study. In and out patients of Pediatric, Azadi Teaching and Kirkuk General Hospitals were included. The sex, age and the residential area of all patients were recorded.

CL screening**:** an air dried smear from the exudate or blood or re-aspirated saline injected to the ulcer was done on and glass slide. The smears were fixed in absolute methanol, stained with undiluted Leishman stain for 3 minutes. Then same amount buffered water (PH6.8) was added and gently shake for 10-12 minutes. The smears were washed off, air dried and viewed under microscope 100x [12, 13].

VL screening:

Sera collection: About 2-3 ml venous blood was drawn carefully form patient whom the physician suspected to have VL and had symptoms like hepato-splenomegaly, lymphadenopathy, fever, anemia and few other symptoms. The blood was transferred into disposable tube, the specimen was left to clot then centrifuged to separate clear serum. The sera were kept at (-20˚C) till used [12].

Visceral leishmaniasis kit: The Kala-azar dipstick rapid test is a immunochromatographic assay for qualitative detection of antibodies to *L. donovani* in human serum. The serum sample when add

will reacts with the dye conjugate coated in the test device. Presence of the red line indicates a positive result while its absence indicates a negative result.

Leshmania vector: The team of Kirkuk Health Office were investigated the presence of different kinds of vector diseases using specialized traps and tools, in nearly all regions of Kirkuk city. Samples of trapped insects from all regions were taken from them and brought to our laboratory for microscopic examination and sand fly detecting in regions of Kirkuk city. Sand fly detection was confirmed by entomologist.

Statistical Analysis: T test in style of homogeneous were used manually, where it was necessary. The significant level used was $P< 0.01$ or 0.05.

Results

A blood sample Figure-1 or re-aspirated saline from CL lesion Figure-2 was not reliable as a smear taken by a swab from the exudate of the lesion Figure-3.

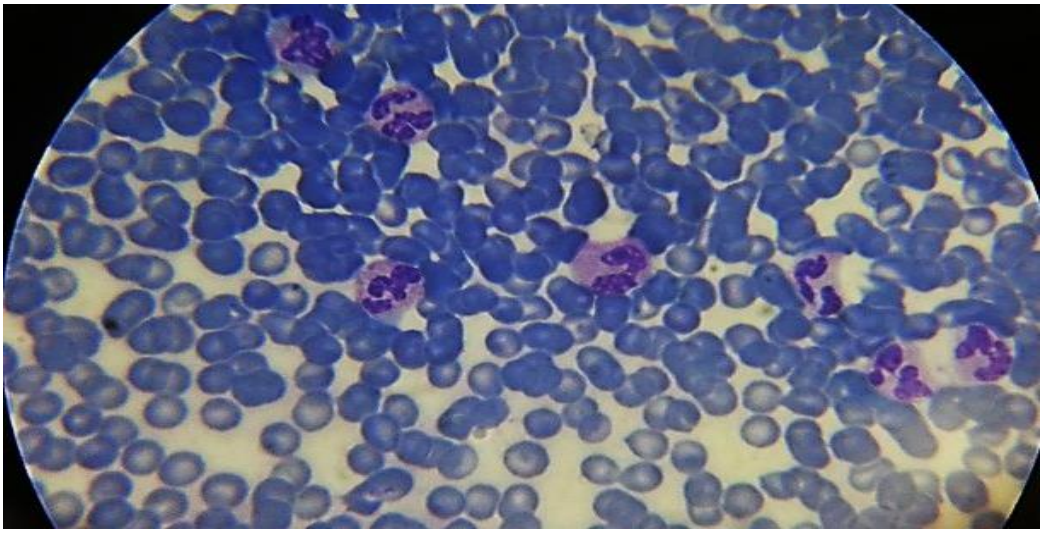

Figure 1-Blood smear from CL lesion showing RBC and WBC cells, (amastigote negative) *Leishman* stain 100x.

Figure 2-CL lesions on leg and hand

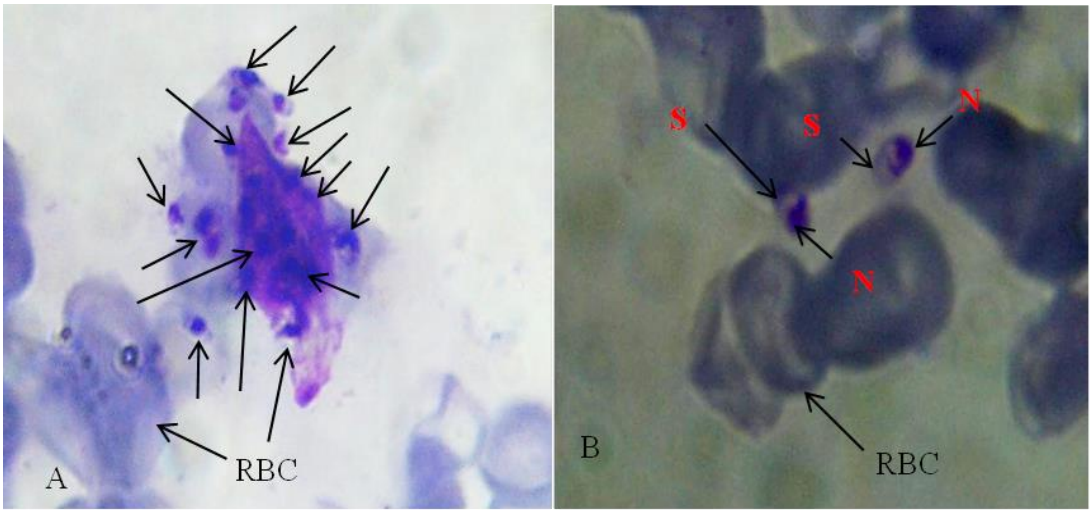

Figure 3- Amastigote stage of *Leishmania* from a lesion swab, A (amstigote inside macrophages) B (two amastigote outside celles), arrows refer to amastigote. N= amastigote nucleus, S= amastigote cytoplasm. *Leishman* stain 100x.

Visceral leishmaiasis patients were chosen depend on their signs as hepato-splenomegaly Figures- (4, 5). The infection detected using dipstick rapid test Figure-6. The rate of VL was much lower than CL, Table-1.

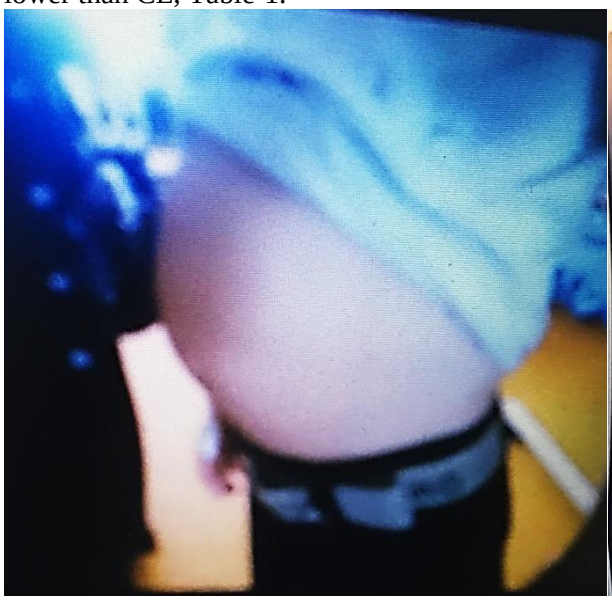

Figure 4-A patient of VL with hepato-splenomegaly.

Figure5-X- ray of a patient of VL with hepato- splenomegaly

The frequency of cutaneous leishmaniasis was significantly much more (64.6 %) comparing with visceral type, where only 3 samples were positive (Figure-6) among 108 suspected person with a rate of 2.8%, Table-1.

Table 1-Incidence of cutaneous and visceral leishmaniasis.

Leishmania type	Total	+ve	%	-ve	%
CL	150	97	64.6	53	35.3
VL	108	3	2.8	105	97.2
Total	258	100	38.7	158	61.2
$\chi 2$ value	Evaluated $\chi 2$ value = 97, $\chi 2$ value of P< 0.005 =7.88 (significant***)				

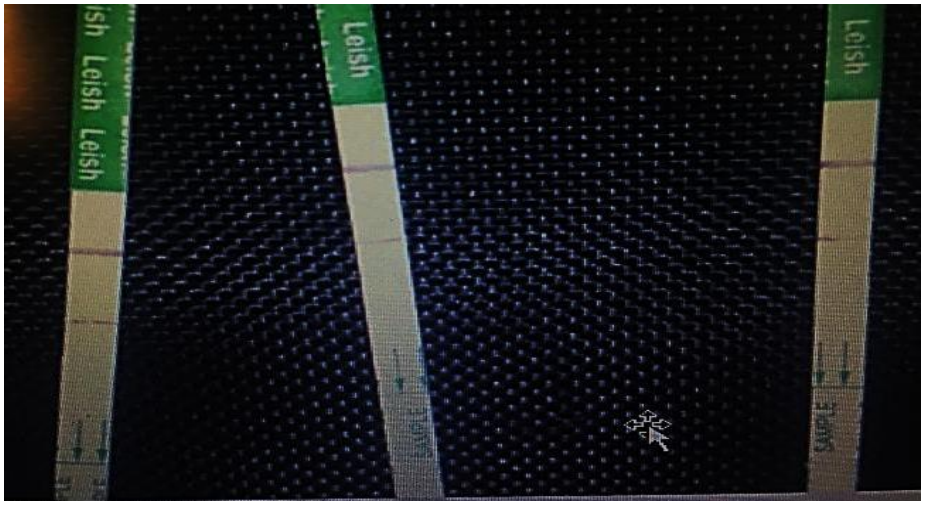

Figure 6-Visceral leishmaniasis positive dipsticks

There were no significant differences between the CL incidence in female and male each with approximately similar rate of incidence, 64.3, 65.2 % respectively, Table-2.

Table 2-Incidence of cutaneous and visceral leishmaniasis according to gender

L.T. / Gender	VL				CL			
	Examined No.	%	+ve %	-ve %	Examined No.	%	+ve %	-ve %
Female	46	42.3	1 2.17	45 97.8	84	56.0	54 64.3	30 35.7
Male	62	57.4	2 3.2	60 96.7	66	44.0	43 65.2	23 34.8
Total	108	100	3 2.8	105 97.2	150	100	97 64.7	53 35.3
χ2 value	Evaluated χ2 value = 1.92, χ2 value of P< 0.01 =6.63 (none significant)							

Table-(3, 4), shows that the small ages are significantly more prone to have the infection comparing with other ages, the higher rates of infection in male were concentrated in ages under 10 years old. The rate of CL was 26.1% among those aged 5≤ years old, and was 16.9 % among those between six to ten years old, Table-3. In female also CL was dominant in ages restricted between few days to fifteen years old. The highest rate was 15.5, 9.5 and 7.1 % in these ages, Table-4.

Table 3-Incidence of cutaneous and visceral leishmaniasis according to male ages

L.T. / Age years	CL				VL			
	Examined No.	+ve %		-ve %	Examined No.	+ve %		-ve %
1 day-5	27	17 26.1		10 15.4	41	2 3.2		39 59.1
6 – 10	16	11 16.9		5 7.7	8	0 0		8 12.1
11 – 15	10	6 9.2		4 6.1	9	0 0		9 13.6
16 – 20	7	5 7.1		2 3.1	2	0 0		2 3.03
21 – 25	2	1 1.5		1 1.5	0	0	0	0 0
26 – 30	1	1 1.5		0 0	0	0	0	0 0
31 – 35	0	0 0		0 0	0	0	0	0 0
36 – 40	3	2 3.1		1 1.5	2	0	0	0 0
Total	66	43 65.2		23 35.4	62	2 3.2		60 96.7
χ2 value	Evaluated χ2 value = 4.5, χ2 value of P< 0.05 =3.84 (significant*)							

Table 4-Incidence of cutaneous and visceral leishmaniasis according to female ages

L.T. / Age years	CL					VL				
	Examined No.	+ve	%	-ve	%	Examined No.	+ve	%	-ve	%
1 day - 5	18	13	15.5	5	5.9	21	1	2.1	20	43.4
6 – 10	10	6	7.1	4	4.7	12	0	0	12	26.1
11 – 15	13	8	9.5	5	5.9	7	0	0	7	15.2
16 – 20	9	7	8.3	2	2.4	2	0	0	2	4.3
21 – 25	6	4	4.7	2	2.4	1	0	0	1	2.2
26 – 30	5	4	4.7	1	1.2	1	0	0	1	2.2
31 – 35	9	6	7.1	3	3.5	0	0	0	0	0
36 – 40	4	1	1.2	3	3.5	0	0	0	0	0
40 <	10	5	5.9	5	5.9	2	0	0	2	4.3
Total	84	54	64.3	30	35.7	46	1	2.1	45	97.8
$\chi 2$ value	Evaluated $\chi 2$ value =5.53, $\chi 2$ value of P< 0.05 =3.84 (significant**)									

Cutaneous leishmaniasis patients having one ulcer was the most dominant type, with rate of 39.1%, followed by patients having two ulcers with a rate of 25.7 % and patients having three ulcers with a rate of 14.4%, Figure-7.

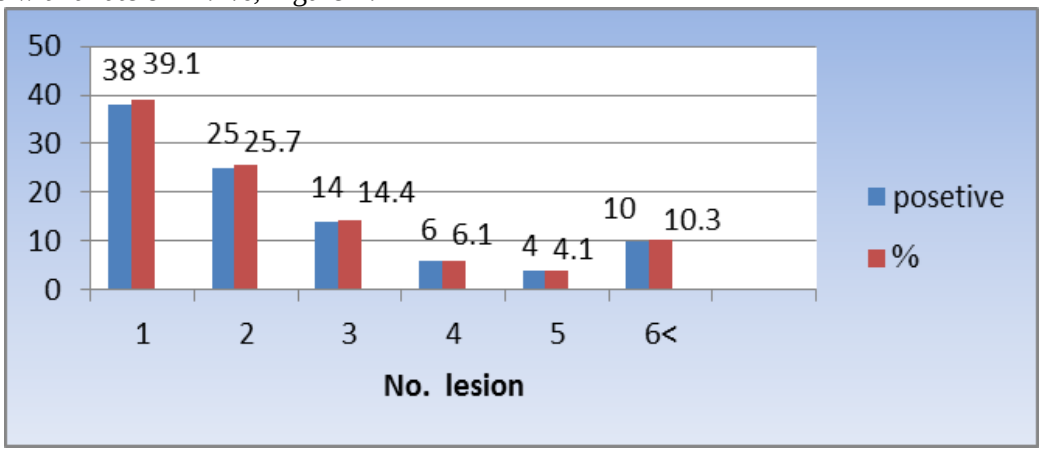

Figure7-Incidence of cutaneous leishmaniasis according to the number of ulcers

The lesion or ulcer type was moist in high percentage of infected patients with a rate of 71.13 % comparing with patients of dry type with a rate of 28.8 %, Figure-8.

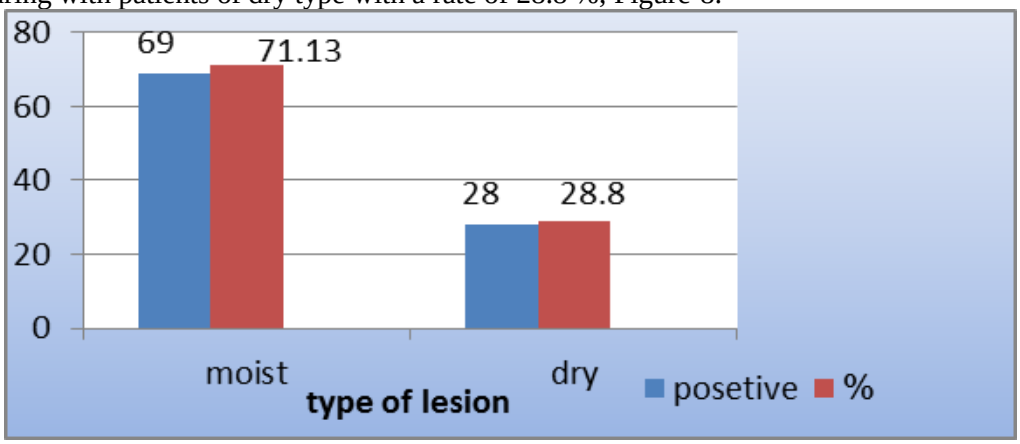

Figure 8-Incidence of cutaneous leishmaniasis according to the type of lesion.

Figure-9 reveals that, upper and lower limbs were highly exposed to the bit of sand fly and formation of lesion or ulcer, with rate of 37.1, 32.9 % for each one respectively, followed by face with rate of 23.7%.

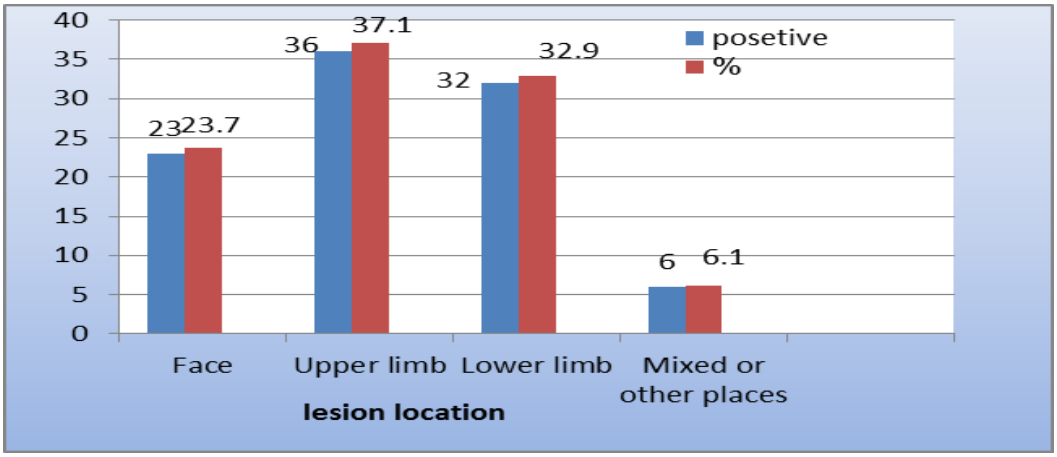

Figure 9-Incidence of cutaneous leishmaniasis according to the lesion location.

The cold months of the year showed the highest rate of CL infection, as it illustrated in Figure- 10, the curve of the infection was raised in November (23.7%), December (25.7 %) and January (17.52%) comparing with the other months of the year.

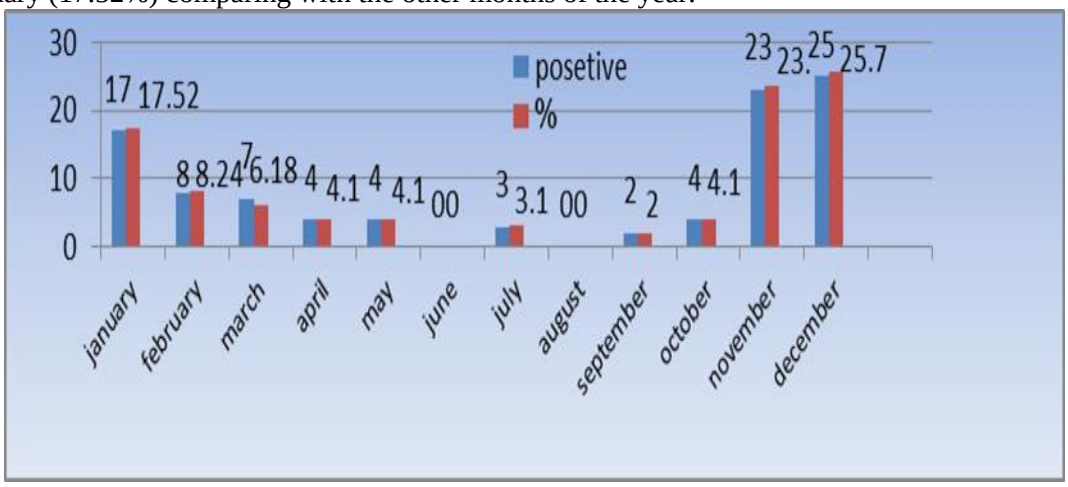

Figure 10-Incidence of cutaneous leishmaniasis according to months.

The frequency of the parasite was a much little higher (52.2%) in environs than in the city center 47.4 %, Table-5. But the highest rate in environs area was Tobzawa with rate of 26.8%, and the highest rate in city center area was Qadisiaah with rate of 8.2 %. And this result was matched to that of vector screening in varied regions of Kirkuk city. The insect or the vector was detected in nearly all regions of the city when collected insect samples examined and viewed under microscope, Figure-11.

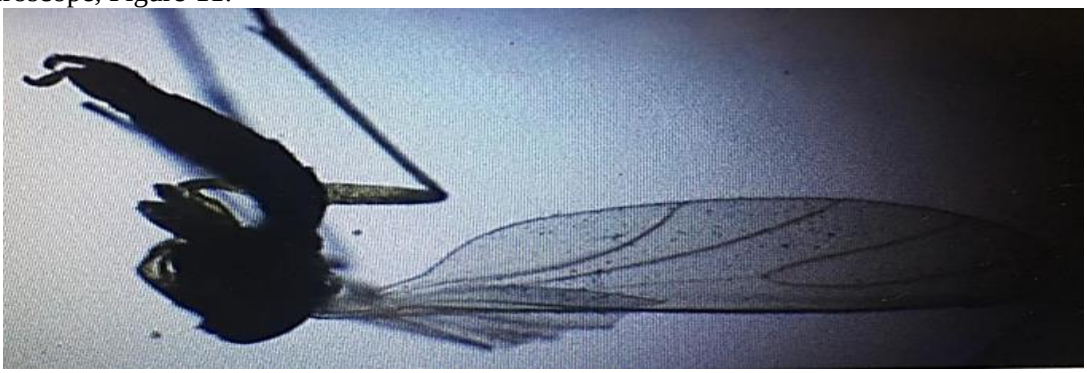

Figure 11-Sand fly female sample detected from Kirkuk city center.

Table 5-Incidence of cutaneous leishmaniasis according to the residential areas

Residential areas				
City center			Environs	
Regions	+ve	%	Regions	+ve %
Xernata	4	4.1	Tobzawa	26 26.8
Qadisiaah	8	8.2	haweja	11 11.3
Hai-nasr	4	4.1	Taza	5 5.1
Hai –al alaskari	1	1.0	Dobbs	4 4.1
Tariq Baghdad	1	1.0	Daquq	3 3.0
Panja ali	6	6.1	Kefri	2 2.0
Shorijh	5	5.1	Total	51 52.5
Failak	4	4.1	----------	----------
Raheemawa	2	2.0	----------	---------
Mosala	1	1.0	----------	----------
Uroba	2	2.0	----------	----------
Ronaki	2	2.0	----------	----------
Escan	4	4.1	----------	---------
1 Huzairan	2	2.0	----------	---------
Total	46	47.4	---------	---------

Discussion:

In incidence of CL and VL in this study, were greatly varied. The visceral type was the less with rate of 2.8 % comparing to cutaneous type with rate of 64.6%. An identical results recorded very low frequency of VL in northern part of our country [11]. In CL infection self-healing can occur after a while, but VL can be fatal if leaved untreated. This result is probably because of that, the species causing VL type is limited to small ages, which was proper for our study, in which only children was infected with VL. Therefore it may be expected that the causative agent is of *L. donovani infatum* or may be the anthroponotic visceral leishmaniasis that caused by *L. donovani* and transmit only by one species of sand fly (*P. alexandri*) with no any reservoir hosts [2,13]. In contrast the CL was found infecting all ages, this will possibly increase its prevalence, in addition to that the vector of this type can live indoors which may amplify the exposer ratio. Certain species, like *Phlebotomus bergeroti, P. papatasi, and P. arabicus*, prefer indoor habitats, while others, like *P. alexandri*, are found in outside environment [14, 15]. Worldwide, it was estimated about 13 million people annually to be infected with CL with an expected increasing rate of 1.2 million [16]. CL, resulted from *Leishmania major*, have been recorded in Afghanistan, Egypt, Iran, Iraq, Jordan, Libya, Morocco, Palestine, Pakistan, Saudi Arabia, Sudan, Syria, Tunisia and Yemen [16, 17, 18]. Most of the achieved studies in Iraq were on CL type, out of 85 suspected cases of CL in Wasit 60 % were positive by direct smear, 85 % were positive on culture and iso-enzyme analysis [6]. Leishmanin test was positive in 179 patients (90.5%) in Baghdad [7]. A retrospective analysis for recorded cases in Iraqi military in 2000, indicated the presence of CL with a rate ranged from 8.69-65.2% [8]. Of 23,778 persons in Alhaweja, Kirkuk, 107 were positive for cutaneous leishmaniasis with an incidence rate of 45 cases per 10,000 [5]. This fluctuation in parasite prevalence may be due the differences in sample size, or due to sampling time and/or to vector breeding time.

The small ages in this study, were significantly more prone to have the infection, comparing with bigger ages in both male and femal. No significant differences had appeared in CL incidence between males and females. Similar results were found by others, approximately 50% of patients infected worldwide were children [15]. Rahi, 2013, revealed a rate of 56.5, 43.5 % in small aged male and female respectively, with no significant differences between genders [10]. The rate of the disease were higher in years under 15 in both male and female [5]. This may because of the adaptive immunity against the disease in big ages due to the possibility of continuous exposer to the parasite during their life time. In current study, cutaneous leishmaniasis patients having one ulcer was the most dominant type, with a rate of 39.1%. Followed by patients having two ulcers, with a rate of 25.7 %, and patients having three ulcers, with a rate of 14.4%. This accorded with

results found by other studies [5-8].The lesion or ulcer type, was moist in high percentage (71.13 %) of infected patients, comparing with patients of dry type (28.8 %). CL lesions were more prominent on upper and lower limbs, with rates of 37.1, 32.9 % respectively. Followed by face region with rate of 23.7%. Similar result found that, 36.5% of CL infected patients had dry lesions while 63.5% had wet lesions, and that 57% of the lesions were on upper limbs, 25% on face and 15% on lower limbs [5]. Comparable to this, was what found by Dhia El-Deen and Abdulah, 2006, whom revealed the lower limb (58%) as most common site of infection followed by the upper limb (24%), and the head and neck (18%) [8]. Also Rahi, 2011, confirmed that face, upper limbs and lower limbs are site of high rate ulcers [6]. The high frequency of moist ulcer, may be because of *L. tropica major* which cause wet or rural cutaneous leishmaniasis, that transmitted by *P. papatasi* sand fly and many rodent species of *Psammomys obesus*, *Meriones libycus*, *Nesokia indica*, and *Rhombomys opimus* serve as its reservoir hosts [2]. While *L. tropica minor* which cause dry or urban cutaneous leishmaniasis is anthroponotic with no reservoir hosts [13].

The cold months of the year recorded highest rate of CL infection, the infection was raised in November (23.7%), December (25.7 %) and January (17.52%) comparing with the other months. This was agreed with what found by others [5, 8, 12]. This may possibly because of vectors biology and its activity in these moths of the year. The sand fly survive at temperatures 16-44 C, therefore they are mostly found between the months of May to November [13, 14]. The frequency of the parasite was higher (52.2%) in environs than in the city center 47.4 %, this was agreed with [5]. All regions of the city center was recorded to have infection, which was corresponded to vector prevalence. Many aspects may influence the presence and distribution of CL in any district, like the reservoir hosts presence, abundant of water areas and rocks, the preferred insect breeding sites, population works in agriculture and spend long time outdoors. Therefore it's better to do serious steps in order to prevent *Leishmania* distribution by controlling vector, keeping reservoirs hosts away from human residence, proper treating of infected persons.

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