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A survey study for prevalence of visceral leishmaniasis in Karbala, Iraq

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Abstract--Visceral leishmaniasis (VL) is a parasitic disease transmitted by biological vectors and caused by infection with the parasites of *Leishmania donovani* complex. After malaria, VL is the second most deadly parasitic disease in the world if not treated early in the infection, A survey study was conducted that included 252 people suspected to having visceral leishmaniasis, were reviewed in hospitals of Karbala governorate at Al-Hussein Teaching Hospital, Al-Hindiya Hospital, Ain Al-Tamr General Hospital, Al-Hussainiya Hospital, and Children's Hospital during January 2021 to June of the same year, After diagnosing cases by doctors specializing in pediatric and internal diseases, as the diagnosis was based on the bone marrow examination of patients, out of 252 suspected cases of visceral leishmaniasis, 17 individuals were confirmed to have VL leishmaniasis distributed among the above-mentioned hospitals, the current study reviewed the category data Age, gender and prevalence areas by geographical area and months of infection, The results proved that the highest rates of infection were in the months of January, February, April and June, with percentages of 0.2%), 0.2%, 0.4%, 0.2% (respectively, with an average general rate for months that reached (3.52%), and that the highest Injuries according to geographical area were recorded in the Children's Specialist Hospital of the Center Sector of Karbala Governorate with a percentage of 8.24%) with a general average rate of total injuries for the areas covered by the

research 6.74%, , It was also noted that the highest percentage of infection according to the age factor amounted to 7.14% and this percentage is for the age group confined between (9-5 years), and the results showed that male children were affected by the disease more than females, with an average general rate of 1.25%.

Keywords--Visceral leishmaniasis, Karbala governorate, *Leishmania donovani*, parasitic disease, Iraq.

Introduction

Visceral leishmaniasis, also known as kala azar, is transmitted by biological vectors and is caused by parasites of the *Leishmania donovani* complex, which includes the parasites *Leishmania donovani* and *Leishmania infantum*. These parasitic species are often considered to be responsible for the visceral spectrum of the disease (Ramzi and Zghair, 2020), As it is transmitted by the phlebotomy sandfly, it may be human or animal, and this disease is considered the second most deadly parasitic disease in the world after malaria if untreated, and is responsible for an estimated 500,000 cases every year worldwide (Dortay and Mueller-Roeber, 2010). This problem is linked to the emergence of resistance in *Leishmania* strains against most antibiotics used in treatment (Moore and Lockwood, 2010). Leishmaniasis is endemic in the tropics and subtropics (Jenson et al., 2004). In Iraq, the disease represents one of the public and serious health problems, as the disease spreads more in the south and center than in the north of the country, with more infections recorded in the winter, followed by the spring, and lower in the summer (Abid, 2004). In endemic areas, the infection rate decreases with age, which indicates the acquisition of immunity in the population over time. Children are the most targeted age group for infection with this deadly disease in the New World and the Mediterranean region (Al-Warid et al., 2019).

There are three clinical forms of leishmaniasis: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL) (Marquardt et al., 2000). Infection with one of these forms depends on several factors, including the genetic variance of the host and its immune response, as well as on the type of the biological vector of the parasite represented by sand flies, in addition to the surrounding environmental factors and the type of the influencing parasite (Smith et al., 2007). The most affected countries are Bangladesh, Brazil, India, Sudan and Nepal (Harhay et al., 2011; McCall et al., 2013). The disease targets the world's poorest groups (Antinori et al., 2012). This is due to the poor health services that prevent the early detection and diagnosis of the disease, the difficult economic conditions, the high cost and length of treatment, and illegal immigration in search of a suitable place to live, in addition to the large number of wars and conflicts, all of these conditions contribute to the uncontrolled spread of the disease (Gani et al., 2010).

Visceral disease is one of the endemic diseases in Iraq that having a population of more than 36 million people, 23% of whom live below the poverty line. The country has witnessed many conflicts and wars during the past 25 years (Majeed et al., 2013). The development of the disease is a result of the spread of parasitic

species that cause visceral disease within the internal organs such as the liver, spleen and bone marrow, which results in the emergence of various symptoms of persistent systemic infection, including fever, weight loss, anemia and lack of appetite, in addition to enlargement of the spleen, liver and lymph nodes (Awasthi et al., 2004; Cheng, 2012). The disease causes about 70,000 deaths annually, as a result of its toxic effects on the affected human body, severe weight loss, anemia and systemic weakness that leads to the death of the patient if not treated appropriately, knowing that patients who recover from infection with visceral disease acquire immunity for life, but in some cases, an activation state for infection with the parasite can occur if the person suffers from diseases related to the human immune system (McGwire and Satoskar, 2014).

Materials and Methods

Samples collection

The study included 252 people suspected of having visceral leishmaniasis after diagnosing cases by doctors specializing in pediatric and internal diseases in hospitals in Karbala governorate in its districts and districts, which included (Al-Hussein Teaching Hospital, Al-Hindiya Hospital, Ain Al-Tamr General Hospital, Al-Hussainiya Hospital, Children's Hospital) for the period From January 2021 to June of the same year, As it was confirmed that 17 individuals were infected with leishmaniasis VL, distributed to the above-mentioned hospitals, the samples extracted from the bone marrow of the affected patients were placed in ampoules containing the prepared culture media and incubated in the studies laboratory of the Holy Shrine at a temperature of 26°C, which is the appropriate degree for the growth of the flagella stage of the parasite until the procedure Treating it with therapeutic plant extracts, while writing down the full information of patients who have been confirmed to be infected.

Parasite isolate

Traditionally, the visceral leishmaniasis parasite is isolated from the bone marrow of patients, after being diagnosed by specialized pediatricians according to the method (Al-Hussaini et al., 2017). As this process was carried out with the help of the specialized pediatrician, the sample-taking area, which is the pelvic bone in the lower back, was wiped with ethyl alcohol at a concentration of 70% and then left to dry a little. The patient is injected with a simple intravenous anesthetic, then a bone marrow tube is inserted in the form of a thin (hollow) tube into the pelvic bone to withdraw a sample of bone marrow fluid and another from the bone marrow tissue (Hatam et al., 1997; Noyes et al., 1998). By PCR technique, prepared isolates of *L. donovani* parasite were also obtained (Yaseen and Ali, 2016). From the Center for Research and Biotechnology of Al-Nahrain University - Baghdad, as these isolates represented negative control elements, while samples taken from patients represented positive control elements.

***Leishmania donovani* culture**

The parasite with its promastigots was grown and activated in Novy- MacNeal-Nicolle (NNN) medium, then transferred to (RPMI-1640) medium supplemented

with 10% FBS serum and 1% antibiotic (penicillin and streptomycin) under conditions of Very sterile, then placed in a cooled incubator at a temperature of 26 °C. It is the appropriate degree for the growth of the promastigote phase, as this process was carried out in the studies laboratory of the Imam Hussein Holy Shrine, and after 72 hours, the pre-incubated culture media were examined to ensure the growth of the promastigote phase of the parasite by taking a drop from the medium and brushing it on the slide, and then conducting a microscopic examination. For a slide prepared under a 40x lens, when confirming the appearance of the flagella phase of the parasite, 0.5ml of the primary isolate was transferred to another tube containing the same culture medium and of the same size in order to make a sub culture for the parasitic isolate in order to multiply the cells and purify them from contamination, after that the logarithmic phase of the parasite begins, then we will notice Flush phenomenon of clustered parasitic cells (Figure 1).

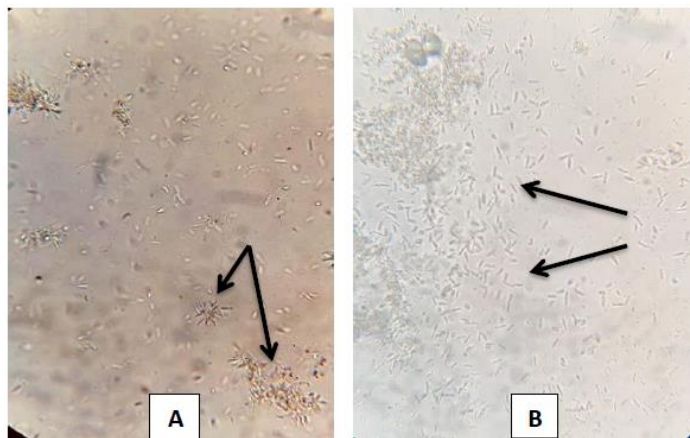


Figure 1. *L. donovani* promastigotes cells under a 40x light microscope) grown in RPMI-1640 medium and incubated in a cooled incubator below 26°C: A/ *L. donovani* promastigotes with rosette shape represents the flushing phenomenon, B/ *L. donovani* promastigotes represents mononuclear cells

Statistical analysis

The statistical analysis program SAS 2012. Statistical Analysis System was used to detect the influence of the different factors in the study parameters, also the LSD Analysis of Variation-ANOVA test was used, the value of the least significant difference for the important comparison between the means used. In addition, SPSS and Excel 2010 were used.

Result and Discussion

Infection rates in Karbala governorate (study areas) according to the months of the year

The results contained in table (1) showed that the highest rates of infection in the months of January, February, April and June were recorded in the Children's Specialized Hospital of the Center Sector, with percentages of 0.2%, 0.2%, 0.4%,

0.2 % respectively, as these values represented the percentage of injuries among male children only, with an average general rate of 1.25%.

Table 1
Represents the infection rates in the study areas during the first months of the year and by gender

Months	Husseini Hospital		Al-Hindia Hospital		Al-Hussainiya Hospital		Ain Al-Tamr Hospital		Childrens Hospital		The total	Chi calculated
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
January	0	1	0	0	0	0	1	0	0.2	0.66	0.29	22.27
February	0	0	0	0.5	0	0.33	0	0	0.2	0	0.17	
March	0	0	0	0	0	0.66	0	0.5	0	0	0.17	
April	0	0	0	0.5	0	0	0	0.5	0.4	0.33	0.29	
May	0	0	0	0	0	0	0	0	0	0	0	
June	0	0	0	0	0	0	0	0	0.2	0	0.58	
Total	0	0	0	0.11	0	0.17	0.58	0.11	0.29	0.17	3.52	
	0.58											
Kai calculated value	1.46											

Chi calculated value 22.27 for the months
Chi calculated value 1.46 for the male and female
Chi calculated value 8.56 for the study areas

when $P \geq 0.05$

As for the month of March, the highest rates of infection were recorded in public hospitals affiliated with the districts of Husseiniya and Ain al-Tamr, with percentages of 0.66% and 0.5%, respectively, knowing that injuries were recorded among females exclusively in the two health facilities mentioned above, while in the month of May they were not recorded. Any infection with visceral disease (VL) in any of the regions under study, The statistical results indicated that there were significant differences between the months of the year in the percentages of injuries, where the calculated chi value reached 22.27, which is greater than the tabular chi value at the level of probability $P \leq 0.05$, While there were no significant differences in the percentages of injuries between males and females, the calculated chi value was 1.46, which is smaller than the tabular chi value at the level of probability $P \leq 0.05$ and the degree of freedom of 1, as well as there are no significant differences in the percentages of injuries between the areas under study, which amounted to the calculated value of Chi(x²) is 8.56 which is less than the tabulated value of Chi (x²) at a probability level of $P \leq 0.05$ and a degree of freedom of 4.

Table 1. Indicated the presence of visceral leishmaniasis (VL) infections in the central sector of Karbala governorate and for females in particular without males during the month of January, with a percentage of 1%, which represents the highest infection rate for females, while there were no injuries for the sample. The same studied in the rest of the months of the study, as the lowest

percentages of 0% were recorded for the other months alike, while no infection was recorded for males during the mentioned month or the other months under study in Al-Husseini General Hospital, and through the results we note in general that there is no correlation in incidence rates between males and females, as shown in the figure below:

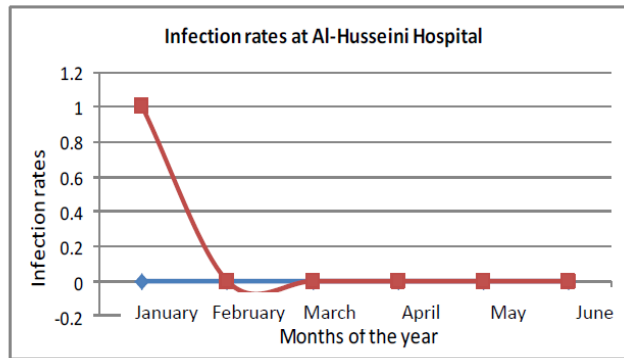


Figure 1. shows the infection rates in Al-Husseini General Hospital, Male ■, Female ■

Figure 1. The infection rates in Al-Husseini General Hospital, Male, Female Looking at the rates of injuries in the Hindiya region, we will notice from Figure (2) that the highest rates of infection were recorded in the months of February and April for females as well, with percentages of 0.5% and 0.5%, respectively, while no injuries were recorded among females in the rest of the months of the study. The percentage was 0%, and no infection was recorded among males in Al Hindiya General Hospital during the months under study, and from the above it is clear that there is no correlation in the infection rates between males and females as shown in the figure below:

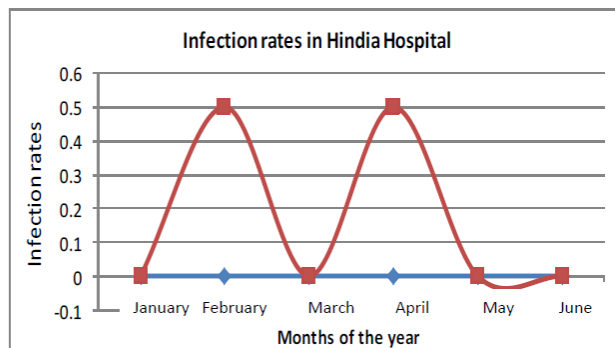


Figure 2. Infection rates in Hindi Hospital, Male ■, Female ■

In comparison with the results of infections in the Al-Hussainiya district, we will notice from Figure (3) that the highest rates of infections with visceral parasitic disease (VL) were recorded in February and March among females as well, with percentage values of 0.33% and 0.66%, respectively, while female infections were recorded. The lowest percentages amounted to 0% in the rest of the months under

study, while no infection with visceral VL disease was recorded for males during the months studied and for the same mentioned regions shown in the figure below:

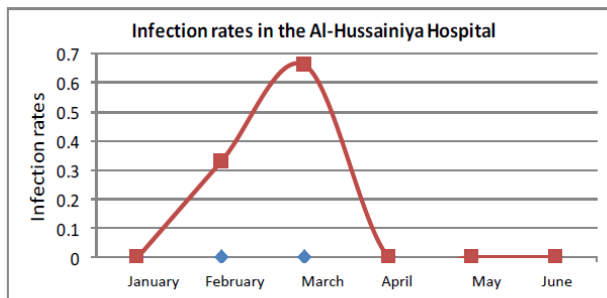


Figure 3. Infection rates in Al-Hussainiya Hospital , Male ■ , Female ■

The results shown in Figure (4) showed that the Ain Al-Tamr region had recorded infections with the visceral parasite for both males and females at different time intervals. % where this value represented the highest percentage that was recorded during the month of January for the male category, while no infections were recorded for the same sample during the rest of the months under study, and for the female category, cases of visceral disease were recorded during the months of March and April, and that With a percentage of 0.5% for each of the two months mentioned above, while Ain Al-Tamr General Hospital did not record any infections for the female category in the remaining study months, January, February, May, and June, From the foregoing, it is clear that there is no correlation in the incidence rates between males and females.

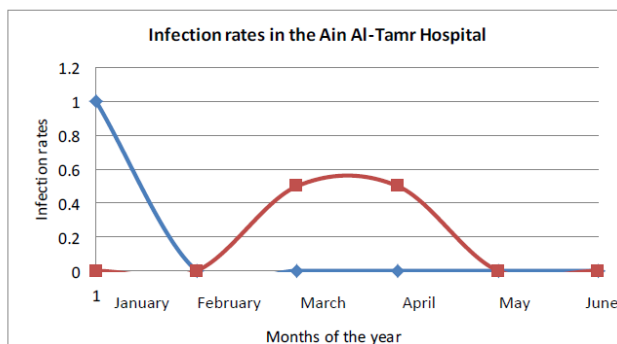


Figure 4. Infection rates in Ain Al-Tamr General Hospital. Male ■ , Female ■

Whereas the results shown in Figure (5) indicated that the General Children's Hospital of the Center Sector in Karbala governorate had recorded sporadic infections for both male and female groups at different time intervals in the first months of the year, where the incidence of visceral disease among males was the highest. than in females, with a percentage of 0.2% for each of January, February and June, respectively, while in April the percentage of infection was 0.4%, where the latter tripled the highest percentage of male injuries Within the

area under study, the Children's Hospital did not record any significant injuries for both males and females during the period of March and May, as the lowest percentage of 0% was recorded during this period for the two concerned groups. As for female injuries during the month of January, the highest percentage was recorded among the ranks of these category during the same month, with a rate of 0.66%, and then a percentage of 0.33% During the month of April, and thus the second highest rate of infection for females, noting that the months of February and June did not witness any infection with the visceral parasite for females. From the foregoing, it is clear to us that the parasite infects males at a higher rate than females, and this is what agreed with the study (Mohebali, 2013), and study (Al-Ani et al., 2012), Where the researcher mentioned that the rates of infection with Kala-azar disease are more active in males than females, and this may be the reason for the lack of movement and mixing of females, and consequently males are exposed to flies bites more than females, and this is what agreed with the study (Kumar and Nylén, 2012).

We also note from the results shown in Table (1) that the highest rates of infection with the visceral parasite were observed in the months of January and April, and this was in agreement with the study (Al-Ani et al., 2012), and study (Rahi et al., 2013), The reason may be due to the season of sand flies infestation, where they are active during the summer and infectious bites occur in the period between June to October, but clinical symptoms begin to appear after an incubation period ranging from several weeks to months, i.e. with the beginning of spring and winter, and this is what the study agreed with (Aoun et al., 2013).

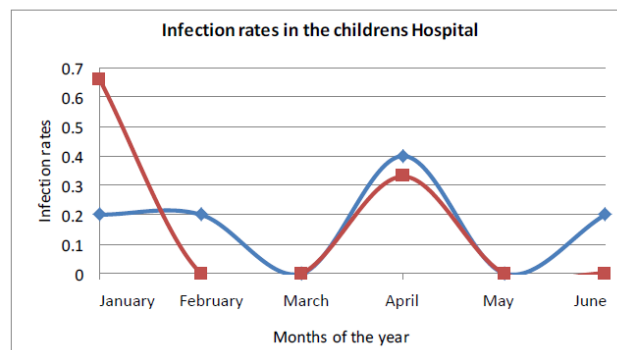


Figure 5. Infection rates at Children's Specialized Hospital, Male ■, Female ■

Returning to the previous results, we will find that Al-Hussein Medical Hospital had the lowest rates of infection compared to the rest of the regions under study, especially the Children's Hospital, which had occupied the first place in terms of the number of recorded injuries, and the reason for the low number of injuries recorded in the Hussein General Hospital may be due to awareness. The residents of the province have the specialty of the Specialized Children's Hospital and the extent of its readiness to receive health conditions for children of all kinds, in addition to the auditors' knowledge that the Children's Hospital has a specialized medical staff to treat the health symptoms of this age group, especially

since the two health facilities are located within the same center sector of the Karbala governorate.

The spread of infections in the areas under study

The results shown in Table (2) showed the different rates of visceral disease (VL) infections, according to the geographical area of the areas highlighted by the current study, as the infection rates ranged between 1.92% in Al-Hussein Medical Hospital and 9.09% in Al-Husseiniya General Hospital of Al-Hussainiya District, while The other regions represented by the Al-Hindiya General Hospital of the Al-Hindiya district, and the Ain Al-Tamr Hospital in the Ain Al-Tamr district gave percentages of 6.45% and 7.69%, respectively, while the Children's Specialist Hospital affiliated to the Markaz sector of the Karbala governorate gave a percentage of 8.24%, and the rate of injuries reached The total area covered by the research is 6.74%.

Table 2
Infection rates for the areas under study

Study areas	Sample total	Infected	Percentage of infection	Chi calculated
Al Hussein Hospital	52	1	1.92%	1.69
Al Hindiya Hospital	31	2	6.45%	0.57
Ain Al-Tamr Hospital	39	3	7.69%	0.04
Al- Hussainiya Hospital	33	3	9.09%	0.04
Childrens Hospital	97	8	8.16%	6.22
Total	252	17	6.74%	8.56

Chi-scuer(x^2) tabular 3.841 , when $df = 1$, and probability level = $P \leq 0.05$

The results shown in the above table indicated that the total rate of infection rates reached 6.74%, which is a small percentage compared to the results of the studies conducted, where it was mentioned(Rahi et al., 2013),That the infection rate in Baghdad governorate amounted to 48%, while in Anbar governorate, the researcher mentioned(Al-Ani et al., 2012), That the incidence of visceral disease was 2.6%, which is considered a small percentage compared to the results of the current study, and in Maysan, a similar study showed that the rate was 3.5%(Alkaisi et al., 2015),The statistical results indicated that there are no differences in the incidence rates according to the regions under study.

Distribution of infection rates according to age group

It is evident from the results presented in Table (3) that the percentage of injuries varies according to the age group, as it was noted that the highest percentage of injuries reached 7.14%, and this percentage is for the age group limited to (9-5 years) compared to the lowest percentage of 6.34% Which was for the age group

confined between (14-10 years), while the younger age group recorded in the number of years, which ranged between (4-1 years), a percentage of injury with a value of 6.59% and it is close to the percentage that preceded it ,as shown below:

Table 3
Percentage of injuries varies according to the age group

age group	Sample total	Infected	Percentage of infection	Chi calculated
1-4	91	6	6.59%	0.028
5-9	98	7	7.14%	0.35
10-14	63	4	6.34%	0.45
Total	252	17	100%	0.828

Chi-scuer (χ^2) tabular 3.841, when $df = 1$, and probability level = $P \leq 0.05$

Looking at the results of the current study mentioned above, we note that it does not agree with the study(Mniouil et al., 2017),Where the researcher indicated in his study that the incidence rates increase in the age groups between (4-1 years) and this is also what was agreed upon by an approach study(Alkaisi et al., 2015), In other studies, the incidence rates differed, depending on the difference in the age groups studied in the study(Collin et al., 2004),The researcher indicated that the age group (9-5) had recorded an injury rate of 9.9%, as this percentage is close to the results of the current study and it agreed with the results of the study(Moheballi, 2013), and study (Bora, 1999),Where the researcher confirmed that the rates of infection appear among children aged between (9-0 years) in particular, and the results of the statistical analysis showed that there are no significant differences between the differences in the rates of infection according to the age groups that fall within the research circle of the current study. The reason for the increase in the rate of infection for the age group (9-5 years) is to increase the number of hours of outdoor play and the frequent mixing and thus exposure to the bites of carrier flies more than in other age groups.

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