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Diagnostic and Epidemiologic Features of Visceral Leishmaniasis Transmission by The Phlebotomus Fly in Children Admitted to The Children's Hospital in Benghazi

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الملخص:

يعتبر داء الليشمانيات عدوى طفيلية ينتقل عادةً بين الحيوانات والانسان عن طريق لدغة أنثى ذباب رمل الماص للدم، وقد أجريت هذه الدراسة لتسليط الضوء على مرض اللشمانيا الحشوي في الاطفال. حيث يُعد ظهور هذا المرض مصدر قلق خوفا من انتشاره بصورة كبيرة، وهو مستوطن في مناطق معينة في ليبيا. استند تشخيص داء الليشمانيا الحشوي على تاريخ المريض والتشخيص السريري، وتم تأكيده بأخذ خزعة من السائل الشوكي لكشف الشكل السوطي للطفيل (Amastigotes)، وتُفحص مجهرياً بعد صبغ المسحة بصبغة جيمسا(Giemsa) حيث تعتبر هذه الطريقة نهج الخط الاول في تشخيص مرض اللشمانيات.

بالإضافة إلى دراسة مقطعية مستعرضة أجريت في الفترة من 1 يناير 2013 إلى 31 ديسمبر 2019 للتحقيق في العوامل الوبائية لمرض داء الليشمانيات الحشوي لدى الاطفال الذين تم إدخالهم إلى مستشفى الاطفال في بنغازي، ليبيا.

أشارت النتائج إلى أنه خالل فترة الدراسة كان هناك 51 طفل مصاب بمرض الليشمانيا الحشوي من بين 99 حالة اشتباه. وكانت الاعراض السريرية الاكثر هي نقص الكريات الحمر وتضخم النوى وتضخم الكبد والطحال والحمى المطولة. ونوع الليشمانيا الذي يسبب المرض هي لشمانيا دونوفاني (Leishmania Donovan) وقد وجدنا الفئة العمرية من 0 -3 سنوات هم الاكثر تضررا ما بين الاطفال، واظهرت نتائج الدراسة زيادة اصابة الطفال الذكور مقارنة بالأطفال الاناث 2 :1، جغرافيا أعلى إصابة كانت في مدينة مرزق الريفية 5.45 ٪ وقد خلصت الدراسة إلى أن داء الليشمانيات يشكل خطر صحي ووبائي في عدة اماكن في ليبيا حيث يصيب الاطفال والبالغين. وسيكون من المهم أن نفهم بعناية كيفية انتقال المرض بين الاشخاص، وزيادة الوعي المهني والمجتمعي، وتحسين التشخيص، وتوافر طرق العالج المناسبة.

الكلمات المفتاحية:

داء الليشمانيا الحشوي ، الاطفال ، مستشفى الاطفال ، بنغازي ، ليبيا.

Abstract

The Leishmania species are the causative agents of leishmaniasis, which is typically transmitted between vertebrate hosts through the bite of blood-sucking female phlebotomine sand flies. This study was conducted due to the paucity of information in the literature relevant to visceral leishmaniasis (VL) in Libyan children. The emergence of visceral leishmaniasis is of concern in Libya and is endemic in certain regions. Diagnosis of visceral leishmaniasis was based on patient history, physical results, and then confirmed by laboratory bone marrow examination. A cross-sectional, hospital-based study was conducted from the 1st of January 2013 to the 31st of December 2019 to investigate the epidemiological factors of VL in children admitted to the Children Hospital in Benghazi, Libya. The results indicated that during the study period there were 51 children diagnosed with VL among 99 suspected cases. The most common clinical symptoms were myelopoiesis (90.9%), aspiration (90.9%), cellularity (89.9%), erythropoiesis (88.9%), megakaryopoiesis (88.9%), hepatosplenomegaly (58.6%) and prolonged fever (48.5%). Children in the age group ≥3 years were most affected, and the male-female ratio was 2: 1. The highest number of cases of children were from the rural city of Murzuq at 45.5%. The study concluded that VL is an endemic health threat affecting children and adults in Libya. Considering that a portion of locally identified Leishmania donovani strains possess the ability to vascularize, it will be important to carefully understand the characteristics of transmission, increase professional and community awareness as well as improving prognosis and the availability of appropriate methods of treatment.

Keywords: visceral leishmaniasis, children, the Children's Hospital, Benghazi, Libya.

1. INTRODUCTION

Visceral leishmaniasis (VL), or kala-azar "Black fever", is a vector-borne disease and the most serious form of leishmaniasis. It is caused by the protozoan parasites Leishmania donovani and L. infantum. VL is transmitted to humans through the bite of infected female sand flies, genus phlebotomus [1]. In the past decade, the number of leishmaniasis cases has increased, both in endemic countries and all over the world [2]. Recent estimates showed about 50 000 to 90 000 new cases of VL. Only about 25–45% of cases which occur each year worldwide are reported to the WHO [3]. This increase may be associated with the increased frequency of travel, ecotourism activity, military operations and immigration, according to an Italian study [4].

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Since VL is a fatal disease when left untreated, and because treatments used have high toxicity, a very sensitive and specific diagnostic test is required. The gold method for diagnosing VL is fu ndamentally the demonstration of the parasite by microscopic examination of splenic aspirate culture. Spleen aspiration can be associated with hemorrhage, and the operation should only be done with access to surgical facilities. For this reason, the most common clinical samples used for this purpose are bone marrow and lymph node aspirates for parasitological diagnosis. The specificity of these methods is high, but the sensitivity differs depending on the type of specimen [5, 6, 7]. In Libya, VL was identified from north-eastern Libya and southern Saharan and sub-Saharan areas where many of the reported VL patients had traveled outside of Libya and the majority were young children [8, 9]. In these areas, there are two endemic foci of infantile visceral leishmaniasis (IVL), caused by the pathogen L. infantum, MON-1. [10]. IVL infects children under 5 years, in Al-Jabel Al-khaddar (northeastern region) and Ubary (southern region) [8, 9, 11]. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia [2].

The exact prevalence of VL in Libya was unknown since most scientific research focused on cutaneous leishmaniasis in terms of the epidemic [12], Spatiotemporal analysis [13] and vector surveillance [14] compared to visceral leishmaniasis, where few papers dealt with the diagnosis, epidemiology, and treatment of the VL disease.

Therefore, this study aimed to shed light on the diagnosis used to recognize the disease in Benghazi's Children's Hospital, where many cases who were less than 15 years old came from various eastern and southern cities.

As well as knowing the spread of the epidemic and the number of cases during the five-year study period. In addition to the demographic characteristics of all cases, the clinical evaluation of patients and the treatments that are given and follow-up cases were investigated.

2. MATERIAL AND METHODS

2.1. Patients and samples

Clinical specimens were obtained from 99 patients, clinically diagnosed with visceral leishmaniasis admitted to the Children's Hospital in Benghazi, the main hospital and reference for the treatment of VL and other diseases in northeastern Libya. Between 2013 and 2019, clinical features and laboratory findings related to children were examined.

Permission for conducting this study was obtained by the Human Resources Department in the Children's Hospital administration, which is responsible for research and studies conducted within the hospital.

2.2. Patients and epidemiological information

Demographic data for the children was recorded, such as age, gender, nationality, usual residence, medical history and diagnostic year, along with the assumed country/region in which leishmaniasis was contracted. Laboratory finding such as an increase in erythrocyte sedimentation rate (ESR), alanine transaminase (SGPT), and aspartate transaminase (SGOT) and major clinical manifestations such as fever, splenomegaly, hepatomegaly and treatment type.

2.3. Diagnosis of VL disease in children

The diagnostic criteria was the detection of a parasite (amastigotes form) in a Giemsa stained bone marrow smear which is a very specific method and the first-line approach in VL diagnosis [15]. Approximately 0.3 ml of bone marrow was aspirated and each bone marrow sample was processed immediately. The samples were not mixed with anti-coagulant to avoid morphological artifacts. The sample was then air-dried, fixed in methanol, and stained with routine stains such as Giemsa or Leishman stain. The diagnosis is considered confirmed if at least one laboratory test is conducted.

2.4. Statistical analysis

The data was analyzed using SPSS Version 21 and Excel 2013

3. RESULTS AND DISCUSSION

The confirmation of cases of VL in Benghazi's Children's Hospital took place mainly by laboratory criteria. Table 1 shows the number of cases during the study period and the number of laboratory-confirmed cases. Among the 99 patients that had a diagnosis of VL in their clinical records, bone marrow examination showed that Leishmania cells were found in only 51 of these patients. A direct examination of the bone marrow shows that all positive cases of VL were infected with the L. donovani parasite (Table 1). It is fairly well established that VL in North Africa and the Middle East is caused by L. donovani and L. infantum. İn Libya, L. donovani was identified as the causative agent of VL a long time ago [16] and this fact was confirmed by our findings.

The most common clinical symptoms of all hospitalized cases and clinically diagnosed VL were myelopoiesis 90.9%, aspiration 90.9%, followed by cellularity 89.9%, erythropoiesis 88.9%, megakaryopoiesis 88.9%, hepatosplenomegaly 58.6% and prolonged fever 48.5% whereas the anemia percentage was only 4% of children figure 1. About half of these cases correspond to Murzuq in southwestern Libya, which had 45.5% confirmed cases in that period (Figure 2).

Regarding the age group, the interval with the highest incidence of cases was ≥3 years (51%), followed by 4 to 7 years (30%) while the lowest infection rate of VL was observed in the

age group between 12 to 15 years. In addition, there are several records that have no information (5%). Based on (Table 2) and (Figure 3), the male to female ratio was 1:2 indicating that there was a statistically significant gender difference. Of the total cases reported, 92.9% were Libyan citizens (Table 2). There was an increase in the number of cases that were cured (42.4%) whereas 7.1% died and 50.5% were negative as shown in Table 2.

The number of VL reports had significant growth over the years analyzed, as shown in Figure 4. There were a few cases observed between 2013 and 2014, and in 2016, a significant increase was recorded, followed by a slight decrease in 2017. The reason for the increase in infection cases may be due to the cessation of the control program that was implemented by the National Center for Disease Control, which was an existing and organized program. This program included reducing rodents and the use of vector control methods by local pest management companies. It was able to eliminate 85-90% of desert mice (Psammomys), which is the animal reservoir in the target areas [17]. After 2011, the role of the National Center became purely technical, providing technical advice to the municipalities where the disease is endemic. Stopping the control program of the transmitters of the disease

meant an increase in their population, and consequently an increase in the infection in the endemic areas, and its spread to new places where the disease had not previously been present.

It is important to note that the decline in the number of cases to zero in 2015 does not mean that there were no incidences of the disease, as in this year the city of Benghazi was in a state of war, and all the cases from the south, which is known to have the highest rate of the disease went to Tripoli for treatment.

The analysis of the monthly distribution for all years of the study of cases of visceral leishmaniasis showed that there are periods with a greater prevalence of the disease. Generally, between October and January is where the highest peaks of the disease are concentrated, especially in December followed by January, as shown in Figure 5.

IVL is known to occur in North Africa [18]. In Libya, there are two endemic foci of (IVL), the northern coastal areas near Tripoli and Al-Jabel Al-khdar (northeastern region) [10]. Most infected cases are under 5 years old. A Tunisian study including 1089 cases revealed that about 50% of VL cases were aged between 1 and 3 years [19], which confirms what we discovered in this study. The sex ratio was male biased as males remain near the animal host for a longer time than females do, however, the difference is not significant. This data is in accordance with previous findings [20]. IVL infects children under 5 years, in Al-Jabel Al-khdar and Ubary (Southern region) [8, 16]. The southern area has reclaimed land for agriculture with increasing water resources and some other environmental changes, which may be partly responsible for creating these new foci [9]. The disease is endemic in South Libya, and rates have been rising due to the country's prolonged crisis. The current study revealed that the majority of cases of children come from the city of Murzuq; similar finding were observed by various authors [21, 22, 23.]. This may be due to complex emergencies and mass population movements, in addition to what was mentioned above about the cessation of control programs in the country.

4. CONCLUSION

Parasitological diagnosis with a demonstration of the amastigote forms in tissues remains the only method of diagnosis used in the hospital. Epidemiologically, it was observed that Murzuq has a considerable percentage of children with VL, which highlights the need for strategies to reduce the disease. After concluding that the vast majority of infected children are from the Southern areas, there is a need for a wider healthcare system so that the patient does not need to travel to get the appropriate treatment, as it is essential that therapy be carried out until the healing process is complete.

There also must be better means of disseminating information so that people can be aware of what the recommendations for prevention and control of leishmaniasis are. The Environmental Protection Agency must also continuously control infected dogs and thus prevent the transmission of the disease to humans.

The data analyzed in this paper regarding VL in the Children's Hospital in Benghazi cannot be extended to cover the whole country, since it depends on the patient's presence in the hospital from various cities and some patients may go to hospitals in the capital, Tripoli. Thus, we point out the limitations of this research.

5. RECOMMENDATIONS

Ideally, the combination of multiple methods is recommended for leishmaniasis diagnosis such as serologic detection of antibodies to recombinant K39 antigen. In addition, Polymerase chain reaction (PCR) is a common molecular technique that is highly sensitive and specific. The control of VL disease depends on focusing on the most common areas, while treatments depend on several factors such as the patient's characteristics (e.g. age, immune status, and kidney/liver function), the extent of disease, availability of drugs, associated infections and previous treatments.

Table 1: Method of identification, causative Leishmania species and study period.

Leishmania spp.	Study period	Suspected cases by clinical examination	Confirmed cases by bone marrow examination	Total number of confirmed cases
L. Donovani	2013	1	1	51
″	2014	2	2	
″	2015	0	0	
″	2016	10	6	
″	2017	19	10	
″	2018	32	17	
″	2019	35	15	

Table 2: Demographic variables of cases admitted to the Children's Hospital

Demographic Variables	Positive	Negative	Total
Age groups			
Miss	1	4	5
≥3	23	28	51
4-7	17	12	29
8-11	7	2	9
12-15	3	2	5
Gender			
Male	33	28	61
Female	18	20	38
Nationality			
Libyan	49	43	92
Non Libyan	2	5	7
Mortality			
Unfollow	2	48	50
Treated	42	0	42
Dead	7	0	7
Total	51	48	99

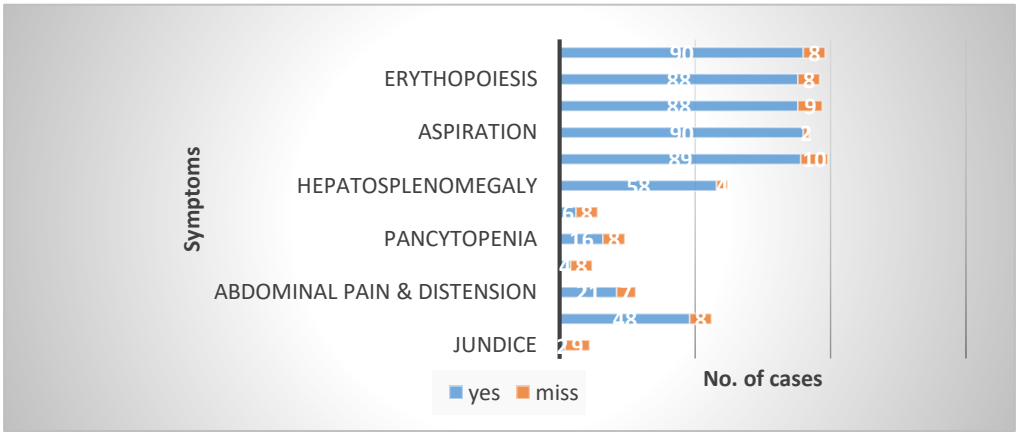


Figure 1. The frequency of clinical symptoms in children admitted with VL to the Children's Hospital between 2013 and 2019.

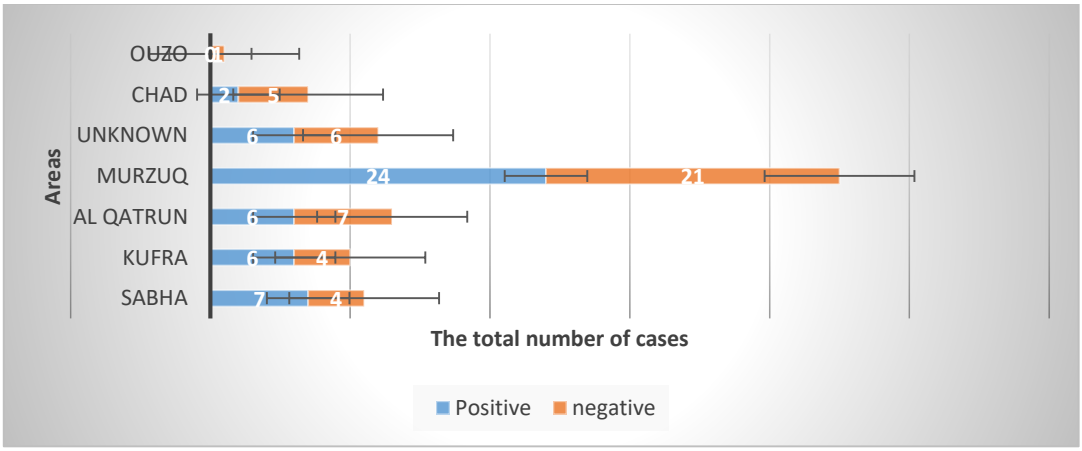


Figure 2: Geographical distribution of children admitted with VL to the Children's Hospital between 2013 and 2019.

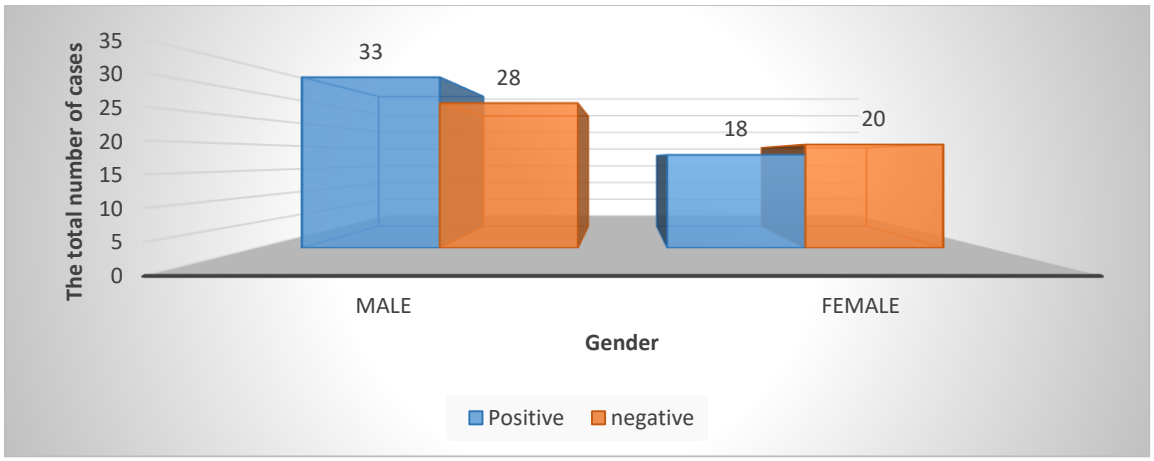


Figure 3: Number of IVL district by gender during the period of 2013-2019

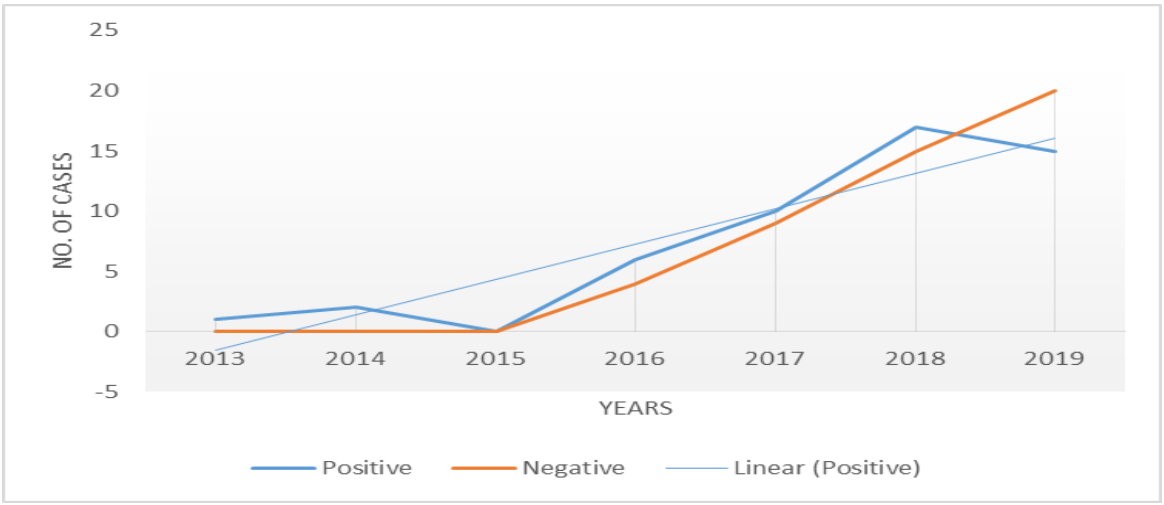


Figure 4: Incidence rate and number of children's cases of VL from 2013 to 2019

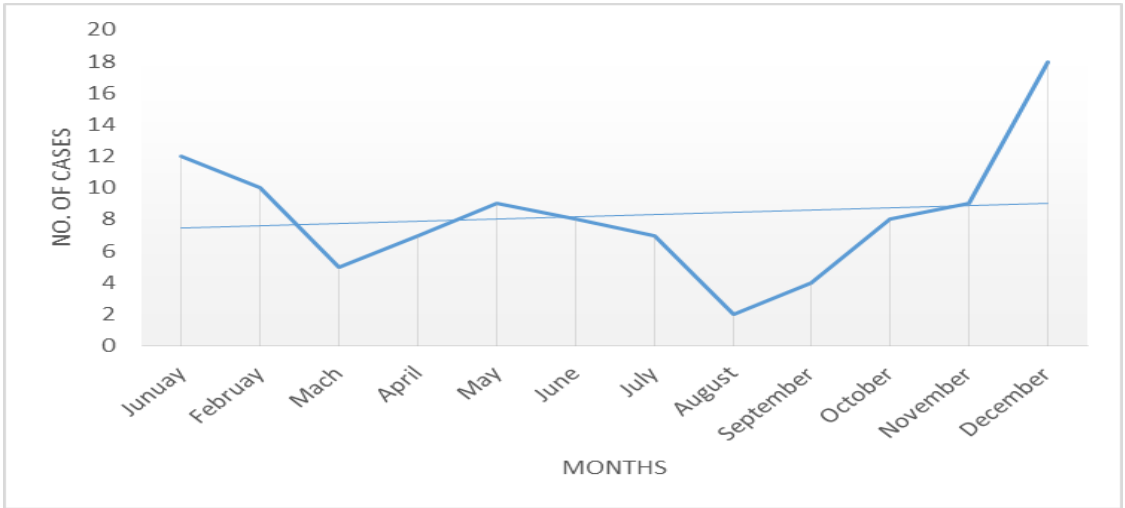


Figure 5: Distribution of confirmed cases of VL from January to December in the years between 2013 and 2019.

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