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Biochemical Assays and Epidemiological Status of Visceral Leishmaniasis among Patient Attending to Benghazi Children's Hospital

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Abstract

According to the World Health Organization Visceral leishmaniasis is a neglected disease and considered a fatal if left untreated. The current retrospective study describes biochemical parameters and epidemiological of 41 VL patients admitted to a Pediatrics hospital in Benghazi, that came from 7 areas distributed in Libyan, the prevalence rate was higher in males 70.7% than in females 29.3%, the majority of the cases (68.3%) were 12 to 15 years old, contributed to Increase in the level of (aspartate aminotransferase), (alanine aminotransferase), alkaline phosphatase, and (C-Reactive Proteins) were seen in 100, 95.1, 85.4 and 36.5% of cases, respectively.

Keywords: Visceral leishmaniasis; *Leishmania donovani*; Bone marrow; C-Reactive Proteins; Benghazi

Introduction

Leishmaniasis is a parasitic disease that is found in parts of the tropics, subtropics, and southern Europe. It is classified as a Neglected Tropical Disease (NTD). The disease is endemic in over 98 countries worldwide and prevalent in areas of the tropics, subtropics, and Southern Europe. More than 90% of global VL cases occur in just six countries: India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia [1]. Leishmaniasis remains one of the major public health problems in the Mediterranean Basin. In Libya, leishmaniases are endemic diseases posing a major threat to public health. Leishmaniasis is a vector-borne disease, which is caused by protozoan flagellates and is transmitted by bite of *phlebotomine* sand flies [2,3]. There are several different forms of leishmaniasis in people. The most common forms are cutaneous leishmaniasis, which causes skin sores, and visceral leishmaniasis, which affects several internal organs (usually spleen, liver, and bone marrow). Although tests that measure the level of serum liver enzymes are commonly referred to as liver function tests, they usually reflect hepatocyte integrity or cholestasis rather than liver function. A change in serum ALT level or AST may be associated with a decrease in liver functioning mass, although neither is specific for liver disease. Clinical and laboratory findings of VL may be different in VL patients in different geographical areas based on the causative agents of the disease. C-Reactive Protein (CRP) is prominent acute phase protein of Human the serum concentration of CRP increases dramatically to nearly 10 to 1000 fold during inflammation following activation of hepatocytes by inflammatory cytokines.

However, the function of CRP in inflammatory conditions and resistance to different infections is still less understood, this may be a part of defense strategies of organism. Accordingly the present study is therefore aimed at assessment and throws more light on the variable of liver, renal function and CRP parameter in the Visceral Leishmaniasis (VL) cases.

Materials and Methods

Study area visceral leishmaniasis was spread in Libya at different places. It has been recorded at the green mountain in the eastern part of the country, also it has been recorded in Tawerqa,

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Misurata, Zawia and the western mountain in the western part of Libyan. As well as in the southern Libyan cities like; Sebha, Algatroon and Murzok. This study has been conducted in Benghazi, Benghazi is the second largest city in Libya, Libya located in North Africa along Mediterranean Sea, neighboring Egypt, Sudan, Chad, Niger, Algeria, and Tunisia.

Study design A retrospective describes biochemical parameters and epidemiological study was undertaken on all new human cases of VL reported in record during 2018 to 2019 of the Benghazi Children's Hospital with signs and symptoms leading to clinical suspicion of VL were included in the study.

Study population we studied 41 patients that came from 7 areas distributed in Libyan southern regions. The patients' ages ranged from 1 to 15 years, the commonest presenting features were fever, abdominal distension and anorexia with weight loss, hepatosplenomegaly and pallor.

Methods

Visceral leishmaniasis suspicion clinical presentation biochemical profile was confirmation and diagnosed on the basis of the history, physical findings and confirmatory laboratory tests and analyzed with peripheral bone marrow smear findings of cases.

Parasitological examination the commonly used method for diagnosing VL has been the demonstration of parasites in splenic or bone marrow aspirate. The presence of the parasite in lymph nodes, liver biopsy, or aspirate specimens or the Buffy coat of peripheral blood can also be demonstrated. In the present study parasitological examinations were performed on suspected cases in humans. Smears were prepared from bone marrow suspected humans. All prepared smears were stained with 10% Giemsa stain solution and examined microscopically for the presence of amastigote forms of *Leishman-Donovan* (LD) bodies.

Biochemical analysis

The estimation of transaminases (ALT, AST and ALP) was done by Reitman and Frankel [4] the optical densities were measured using spectrophotometer.

Statistical analysis

Data entry and data analysis done using Statistical Package for Social Science (SPSS), software version 17. A retrospective analysis was performed and Pearson's correlation coefficient calculated for bone marrow and different variables such as (sex, age, nationality, regions and clinical symptom). Chi-square was used to determine the association of VL disease and demographic or Clinico-Biochemical values by comparing the proportions of any variable in different groups. The significance of correlation between two variables, statistical testing was performed at <0.05 level of significance.

Results

The study included 41 patients proved having visceral leishmaniasis, of which, 37 (90%) were Libyan and 4 (10%) were non Libyan patients.

Table 1: Show Percent (%) of Libyan patients compared with non-Libyan patients.

The study comprised 41 participants; most of them were males 29 (70.7%) while females were only 12(29.30%). The percentage of males was significantly higher (p>0.01) than females as shown in Figure 1.

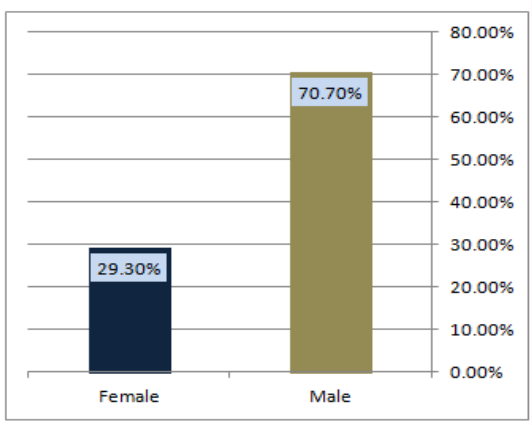

Figure 1: Show distribution of visceral leishmaniasis on gender.

Table 1: Show Percent (%) of Libyan patients compared with non-Libyan patients.

Nationality	N0. of patients	Percent
Libyan	37	90.0 %
non Libyan	4	10%
Total	41	100.0%

Table 2: Illustrate the infection with VL in each age group.

Age	Frequency	Percentage
01-05	4	9.70%
06-11	9	22.00%
12-15	28	68.30%
Total	41	100%

Table 3: The prevalence among patients affected by clinical VL.

Diagnosed clinically	Frequency	Percentage
Hepato-splenomegaly	15	36.60%
Fever +abdominal pain	9	22.00%
Hepato-splenomegaly + Fever + abdominal pain	13	31.70%
Jaundiced + Hepato-splenomegaly + Fever + abdominal pain	4	10.00%
Total	41	100%

Figure 1 show distribution of visceral leishmaniasis on gender.

Mean age of the patients was 5.95 years (± 3.9 years, range, 1 to 15 years).

Highest infections of VL were observed in the age group (12 to 15 yr) as (68.3%), while the lowest infection of VL were observed in the age group (1 to 5) as (9.7%) and the infection rate was significantly higher (p>0.02) in the age group (12 to 15 years).

Table 2 illustrates the infection with VL in each age group.

In fact, this may be due to social behavior of male gender, who are more active out of the houses at night and then more accessible to the vector bits. In general, the difference of gender infection is high statistically significant.

The cases with hepatosplenomegaly symptoms were the most common symptom findings among suspected cases infect with Leishmaniasis is 36.6% followed by hepatosplenomegaly + Fever +abdominal pain, Fever +abdominal pain, Jaundiced+

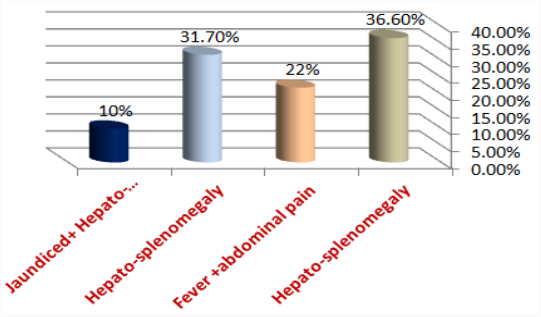

Figure 2: Clinical VL.

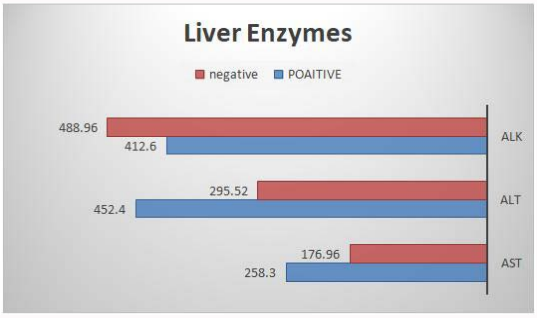

Figure 3:

Table 4: Bone marrow aspirates and macrophage cells features of VL patients.

Bone marrow aspiration	Frequency	Percent
Normal cellularity with Negative (No LD bodies)	26	63.40%
Positive (LD bodies are seen)	15	36.60%
Total	41	100%
Macrophage cells	**Frequency**	**Percent**
Many macrophage cells	16	39.00%
Some macrophage cells	18	43.90%
Non macrophage cells	7	17.10%
Total	41	100%

Table 5: Show Percent (%) of infection According to Libyan district.

Districts	Frequency	Percent %
Al Gatrun	1	2.40%
Alkufra	1	2.40%
Benghazi	2	4.90%
Morzok	21	51.20%
Omalaraneb	1	2.40%
Sabha	11	26.80%
Tchad	4	9.80%
Total	41	100

Hepatosplenomegaly+ Fever +abdominal pain (31.7%, 22%, 10.0%) respectively. The result showed a high significant between symptoms and VL (P>0.001) (Table 3, Figure 2).

The Bone-marrow aspiration has been performed in 41 cases and *L. Donovani*, amastigotes were detected only in 15 (36.6%) of cases. Although the high prevalence of macrophage cells in bone marrow sample has a wide range of implications for diagnosis, but is considered indicative of infected with VL. In this study the patients with some macrophage cells in bone marrow aspiration was high 43.9% followed by patient with many macrophage cells 39.0% while the macrophage cells absence among 17.1% from bone marrow sample patients, bone marrow aspirates and macrophage cells features of VL patients in this study are summarized in Table 4.

Visceral Leishmaniasis (VL), an endemic disease in the littoral zones of the Mediterranean area, the Middle East, East Africa, and especially in Libya, has not been fully documented, the study showed that the most cases were from Morzok district (21 cases, 51.2%) followed by Sabha (11 cases, 26.8%), Chad (4 cases, 9.8%), Benghazi (2 cases, 4.9%) and Algatron, Alkufra, Om alaraneb (1 case, 2.4%) in Table 5.

Table 6: Show mean and STD of liver enzymes in positive and negative bone marrow aspiration.

Bone Marrow Aspiration		
	Positive	**Negative**
AST	258.3 ± 309.9	176.9 ± 107.3
ALT	452.4 ± 901.0	295.5 ± 146.3
ALK	412.6 ± 198.1	488.9 ± 215.9

Table 7: Show mean and STD of renal features of VL patients.

Bone Marrow Aspiration		
	Positive	**Negative**
Urea	20.9 ± 8.6	23.3 ± 8.0
Creatinine	0.7 ± 0.3	0.5 ± 0.2

Laboratory findings revealed anemia in 30 (73.1%) of cases. C-Reactive Protein (CRP) levels were seen in 15 (36.6%) with mean CRP titer 13.36 mg/l, SD ± 20.6 among patients with VL. The CRP levels were higher in patients with bone marrow positive with *L. Donovani* compared with the other groups with VL patients and the Bone marrow negative. On the other hand, patients with VL revealed a significant (p>0.05) increase in the levels of CRP at the time of presentation, an increased level of AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase) and ALP (Alkaline Phosphatase) was found in 41 (100%), 39 (95.1%), and 35 (85.4%), patients, respectively. Alinine Aminotransferase (ALT) was increase in some sever cases reached to 1987 IU/L, while Aspartate Aminotransferase (ATS) so increase and reached to 1397 IU/L (Table 6).

There have been few studies showing kidney function in patients with VL, in this study performed in our region, a total of 41 patients admitted with VL were evaluated. The study showed the no changes in kidney function have been reported among cases with leishmaniasis, no significant differences, also in our study there were no significant correlation of biochemical indices (kidney function) and VL In the serum creatinine (0.7 ± 0.3, 0.5 ± 0.2, P value = 0.36), urea 20.9 ± 8.6, 23.3 ± 8.0 P value = 0.36) (Table 7).

Discussion

To our knowledge, this is the first study to be carried out on the Biochemical assays and Epidemiological Status of visceral Leishmaniasis among children in Benghazi, Libya. The results obtained in this study can provide important information for future understanding of VL infection the World Health Organization (WHO) considered leishmaniasis to be a public health problem in Libya, poor infrastructure in Libya causes leishmaniasis prevalence in most regions. This study was undertaken in clinical and biochemical

features of 41 VL cases, admitted consecutively reported in record of the Benghazi Children's Hospital during period (2018-2019) in Benghazi, city were retrospectively analyzed, the disease was observed year-round, with the highest prevalence between 2018 and 2019.

In the current study sensitivity of bone-marrow microscopy evaluation for detection of *Leishmania* amastigotes was relatively low as parasites were detected in only 36.6% of the bone marrow samples. Bone marrow aspirations have been positive in most (77.5%) of VL patients from Albania and also from Sicily (80.2%) in Italy [5,6]. Bone marrow aspirates are believed to provide a safer but less sensitive method in the diagnosis of Visceral Leishmaniasis (VL) (50% to 85%) compared with splenic aspirate (93% to 98.7%) [7].

The most cases registered were male 29 (70.7%) while females were only 12 (29.30%) this result agrees with Lakhdar Idrissi et al. [8].

The present study shows that the rate of infected children were observed in the age group (12 to 15 yr) as (68.3%) similar results were registered in India and Africa the disease affects older children and adults [9]. In Saudi Arabia, Yemen and Iraq the disease affect infected children less than two years old [10-12].

The Current study revealed that the majority patients had anemia, 30 (73.1%), similar finding were observed by various authors [13-15].

A large spectrum of clinical manifestations accompanies the *Leishmania* attack on reticuloendothelial tissues-liver, spleen, bone marrow, lymph nodes, and the digestive system. The our results showed that the all patients had symptoms range from irregular and recurrent fever, hepatosplenomegaly symptoms were the most common symptom findings among Suspected cases infect with leishmaniasis followed by hepatosplenomegaly-fever- abdominal pain, fever-abdominal pain, Jaundiced- hepatosplenomegaly-Fever -abdominal pain the clinical features of VL in this location were considerably similar to those patients from other parts of the country neighboring countries and even the world [16-18].

The CRP levels were higher 36.6% in patients with bone marrow positive with *L. Donovani* compared with the other groups with VL patients and the bone marrow negative. A positive CRP associated with other signs suggestive of VL, is a good element of diagnosis orientation. This diagnosis is often difficult in front of non-specific clinical signs that are confused with those of other infections (malaria, tuberculosis, brucellosis, enteric fever...) [19,20].

Biochemical parameters are another feature of VL. Increase in the level of ALT, AST, ALP and CRP were seen in VL patients evaluated in this study, because of this, many cases are wrongly diagnosed and treated as hepatitis. We therefore envisaged assessing the actual magnitude of liver function derangement in confirmed cases of visceral leishmaniasis at our hospital. Results in this study the increase in liver enzyme rates seen in this study is consistent with results in other studies of visceral leishmaniasis [21,22].

Conclusion

Visceral leishmaniasis causes functional disturbance in the liver, thus. In some cases, hepatitis confounded and delayed the diagnosis of kala-azar in our study. Early detection of VL cases, prompt treatment and integrated vector management by active and passive case detection approaches through an effective health surveillance system should receive high priority.

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