

## The Sensitivity of fine needle aspiration of spleen for diagnosis of Childhood Visceral Leishmaniasis (Kala-azar) in Yemen

Fadil Abass Abid

Paediatrician, College Of Medicine / University Of AL-Muthanna - Iraq

E.Mail: Falmoktar@Hotmail.Com

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### **Abstract:**

**Background:** Visceral Leishmaniasis is a disease caused by protozoan parasite of the genus *Leishmania donovani*, the parasites migrate to the internal organs such as liver, spleen and bone marrow, and if left untreated, will almost always result in the death of the host. This disease is the second largest parasites killer in the world, and is endemic in Yemen. Visualization of the amastigotes in splenic aspirate is the gold standard for diagnosis of visceral leishmaniasis, this technically challenging procedure is frequently unavailable in the world when visceral Leishmaniasis (VL) is endemic.

**Objectives:** The aim of this study is to confirm the sensitivity of fine needle aspiration of spleen for diagnosis (VL) and the therapeutic responses of patients to sodium stibogluconate (pentostam).

**Materials and Methods:** All cases admitted to the hospital with no specific signs and symptoms, after exclusion of other diseases with the same presentations of visceral Leishmaniasis (VL), I put the suspicion of VL and specific investigations were done like formol gel test, bone marrow aspiration and fine needle aspiration of spleen, the collected data was analyzed and compared with other studies.

**Results:** Twenty patients were studied in children department in Sanaa- Yemen, during June 2007- June 2008, their ages ranged from 3 years to 11 year, all cases from rural areas, presented with fever (100%), Anaemia (45%), anorexia (80%), hepatosplenomegaly and abdominal distension (100%), lymph nodes enlargement (50%), bronchopneumonia (40%), diarrhea (25%) and jaundice (5%). Splenic aspiration was positive in all cases (100%), bone marrow aspiration was positive in (60%), while formol gel test was positive in (50%), all cases responded to pentostam therapy in a dose of 15 mg/kgm single dose daily for 3 weeks intravenous injection slowly (iv).

**Conclusions:** VL was found a common disease in Yemen especially in rural areas affecting children from infancy to adolescence ages, the cause of this disease is well known in addition to its sources like dogs, jackals and poultry farms and the mode of transmission by sand flies, so using of insecticidal agents, destruction of dead animals and using of fine window nets are important measures for the reduction of morbidity and mortality rate of this disease.

**Key words:** formol gel test, bone marrow and splenic aspirations.

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### **I. Introduction:**

Visceral Leishmaniasis is caused by obligate intracellular protozoa of the genus *Leishmania donovani*, it is transmitted from animals like dogs and jackals and poultry farms to human by the vector sand fly of the genus *phlebotomus* which are common in rural areas.<sup>1,2,3,4</sup>

This disease is also called kala-azar or black fever.<sup>9</sup>

It is distributed in many countries, especially Yemen, Iraq, Sudan, India, Pakistan and Bengeladish, and also in Asir region of Saudi Arabia kingdom.<sup>17</sup>

This disease is fatal if untreated because of serious complications that may occur.<sup>5</sup>

Visceral leishmaniasis is acute and resolved if the liver is infected, but is chronic during spleen infection, so it is characterized by splenomegaly and parasitic persistence, the infection will be resolved within 6 to 8 weeks due to development of Th1, then granuloma response will occur, which is caused by the production of IFN- gamma.<sup>6</sup>

Neutrophils, platelets and lymphocytes are all involved in the pathogenesis of *Leishmania donovani*.<sup>20</sup> Patients show marked depressed cellular immune response, as characterized by the absence of delayed hypersensitivity reaction to *Leishmania* antigen.<sup>21</sup> also the inability of peripheral blood mononuclear cells to respond to antigen by either lymphoproliferation or cytokine production.<sup>22</sup>

World widely, the incidence of the disease is 500000 cases per year.<sup>19</sup>

Macrophages are the only cells that in vivo allowed the growth of the intracellular pathogen *Leishmania*, these can also present parasitic antigen to CD4-T lymphocytes known to be involved in protective

and counter protective immune response.<sup>23</sup> parasites in the macrophages cause production of interferon-gama by parasite- specific T- cells.<sup>24</sup>

Most patients admitted to the central hospital with massive hepatosplenomegaly, anorexia, anaemia, fever, lymph nodes enlargement, abdominal distension, bronchopneumonia, and diarrhea.

These signs and symptoms are also presented in other diseases like infectious mononucleosis, malaria, toxoplasmosis, brucellosis, typhoid fever, and leukemia, so these diseases should be excluded, to put the possibility of visceral Leishmaniasis (kala- azar ).

Because of lack of medical staff and facilitated laboratory for diagnosis of this disease and treated properly with pentostam which is not available in rural areas, this is the essential factors to the out break of the disease among these countries and presented with serious complications because of pancytopenia (low haemoglobin, total WBC, and platelets count) and immune system disorder and hypersplenism caused by this disease.<sup>4,12,13</sup>

## **II. Materials and Methods**

Twenty patients were studied in AL-Kuwait teaching hospital, department of children in Sanaa, Yemen. from June 2007 - June 2008, their ages ranged from 3 years to 11 year ( 13 males and 7 females ), with the suspicion of VL.

Clinical examination for detection of hepatosplenomegaly, lymphadenopathy by palpation.

Complete blood count were collected (2ml) in sterile EDTA tube containing anticoagulant agent and the counting done by sysmex system model kx- 21 cell count, Japan (sysmex corporation).

ELISA ( enzyme linked immune sorbent assay) test for detection of hepatitis and liver function test.

Chest X- ray model used is ashimadzu, Japan/ model 06/1.2 p 164/DK-85 for bronchopneumonia and cardiomegaly.

Coagulation studies were done like bleeding time, clotting time, prothrombin time and partial thromboplastin time to exclude bleeding disorder.

General urine and stool examination were done to exclude infection and the causes of diarrhea.

Blood grouping and Rh system used in this study was anti A monoclonal and anti B monoclonal and anti D monoclonal IgG+ IgM (Spanish spin react reagent).

IFAT (immune fluorescent antibody test) for diagnosis of VL, was not available in Yemen during the period of the study.

Bone marrow aspiration from anterior superior iliac crest by using Jamshidi needle, then spread its contents on 5 sterile glass slides, allowed to dry then stained by leishman stain to demonstrate the amastigotes by haematologist.

Formol gel test (formalin gel test), (aldehyde test) which is a test for detection of the greatly increased serum protein in VL: drop of the full strength formalin 40% is added to 1 ml of patient serum with rapid and complete coagulation indicating the positive reaction, this test is cheap and easy to perform but poorly sensitive test bases on detection of polyclonal immunoglobins (from formol- gel test, medical dictionary – the free dictionary- Google).

Fine needle (22 gauge) of 5 ml syringe was used after detection the size of spleen by palpation , I used the Kenyan workers method, so I outlined the size of spleen and inserted the needle only 2 centimeter (cm) depth inside the spleen , 2-3 cm below left costal margin between mid clavicular and anterior axillary lines , after giving valium 0.3 mg/kgm single dose (iv) as sedation to minimize the movement of the patient during the procedure after sterilizing left hypochondrial skin region with iodine and alcohol, then allowed the blood reached to the top of the needle and withdrawal quickly, sterile gauze dressing was applied on punctured area with adhesive plaster, then the needle fixed with syringe to spread its contents on 5 sterile glass slides, now allowed to dry and sent to haematologist for detection of amastigotes of Leishmania donovan bodies.

## **III. Results :**

Twenty patients were the subject of the study, all their residence in the rural areas around Sanaa, the capital of Yemen, their ages were ranged from 3 years to 11 year including (13) males and (7) females.

All these cases were admitted to the hospital for the diagnosis and giving specific therapy, and all these cases were excluded from other diseases with similar presentations to VL . So I put the possibility of VL and specific investigations were done.

All cases (20 patients) were proved to have VL (100%) and presented with fever (100%), anaemia (45%), anorexia (80%), hepatosplenomegaly (100%), abdominal distension (100%), while lymph nodes enlargement (50%), diarrhea (25%) and jaundice (5%).

**Table 1:** Shows the numbers (#), age, sex, haemoglobin (H), total WBC, platelets count and chest x-ray.

#	Age	Sex	Hb( gm/dl)	Total WBC (cells/ $\mu$ l)	Platelets (cells/ $\mu$ l)	Chest x- ray
1	3 years	male	11.8	15000	300000	bronchopneumonia
2	3.5 year	male	12	9000	250000	bronchopneumonia
3	3.8 year	female	11.9	3200	200000	normal
4	4 year	male	9.3	13000	270000	bronchopneumonia
5	4.5 year	male	12	3500	211000	normal
6	4.8 year	male	8.4	3000	180000	normal
7	5 year	female	9.2	15000	270000	bronchopneumonia
8	5.3 year	female	12	3600	210000	bronchopneumonia
9	5.5 year	male	9	2900	95000	normal
10	6 year	male	10.2	3100	157000	normal
11	6.5 year	female	8.7	3000	90000	bronchopneumonia
12	6.8 year	female	12.8	14000	160000	normal
13	7 year	male	13	2800	225000	normal
14	7.4 year	male	9.3	3200	93000	bronchopneumonia
15	8 year	male	12.8	5400	160000	normal
16	8.5 year	female	9	6200	200000	normal
17	9 year	male	12	3200	350000	normal
18	9.7 year	male	9.6	13000	250000	bronchopneumonia
19	10 year	female	11.9	10000	105000	normal
20	11 year	male	12.3	5000	95000	normal

**Table 2:** shows the normal blood count values taken from illustrated text book of paediatrics, UK, London, Tom Lissauer and Graham Clayden/ printed in 1997 – 1998 by Mosby international, page: 323.

Age	Haemoglobin (gm/dl)	Total WBC ( $\times 10^9/L$ )	Platelets ( $\times 10^9 /L$ )
Birth	14.5 – 21.5	10 – 26	150 – 450
2 weeks	13.4 – 19.8	6 – 21	At all ages.
2 months	9.4 – 13.0	6 – 18	
1 Year	11.3 – 14.1	6 – 17.5	
2 – 6 Years	11.5 – 13.5	5 – 17	
6 – 12 Years	11.5 – 15.5	4.5 – 14.5	
12 – 18 Years :			
Male :	13.0 – 16.0	4.5 – 13	
Female :	12.0 – 16.0	4.5 – 13	

**Table 3:** Shows the result of the definitive diagnostic tests of VL in twenty patients.

Methods of diagnosis	# of cases	Percentage
1) Bone marrow aspiration positive and formol gel test positive	8	40%
2) Bone marrow aspiration negative and formal gel test positive	2	10%
3) Bone marrow aspiration positive and formol gel test negative	4	20%
4) Splenic aspiration positive with ( positive or negative Bone marrow aspiration and formol gel test ).	20	100%

**Table 4:** Shows the relationship of VL cases with Blood group (ABO system) of twenty cases.

Blood group	# of patients	Percentage
A	8	40%
B	6	30%
O	4	20%
AB	2	10%
Total	20	100%

#### IV. Discussion:

Twenty cases were studied in al- Kuwait teaching hospital in Sanaa, the capital of Yemen as seen in the figure 3 ( map of Yemen ), during the period from June 2007 – June 2008 among children aged from 3years and 11 year, diagnosed as VL, which is caused by *Leishmania donovani* , transmitted by vector sand fly from animals to human.<sup>1,2,3,4</sup>

The majority of cases were found after the age of 8 months,<sup>7,8</sup> and no cases were reported under the age of 6 months which may be due to the long duration period of the disease that varies from several weeks to several months.<sup>9</sup>

The highest number of cases was found during October, November and December months, because of summer season in Yemen due to the maximum density of the sand flies, while in Iraq and other countries, the disease is common during June, July and August (summer season), when the insect bites occur.<sup>10</sup>

In this study males affected more than females (1.8 ratio), while in other studies both sexes were affected equally.<sup>11</sup>

The signs and symptoms of this disease like fever (100%), anaemia (45%), and anorexia (80%), hepatosplenomegaly and abdominal distension (as detected in the figure 1 and 2 below) account (100%), while lymphadenopathy (50%), bronchopneumonia (40%), diarrhea (25%) and jaundice (5%), were found in this study and also found in other studies<sup>4</sup>.

Complete blood count showed mild anaemia in 9 patients (45%), decreased white blood cells (leucopenia) in 10 Patients (50%) and mildly decreased in platelets count (thrombocytopenia) in 5 patients (25%). Coagulation studies showed bleeding time was slightly prolonged in 5 patients due to thrombocytopenia while clotting time, prothrombin time, and partial thromboplastin time were normal.

General stool exam showed pus cells which indicated the cause of diarrhea was due to infection, while general urine exam was normal, liver function test showed slightly increased in total serum bilirubin only.

The collected data included: patients, ages, sex, residence (rural areas) and simple investigations were done like complete blood count, blood film for morphology, widal test, rose bengal test, toxoplasmosis test and monospot test, chest x- ray, general urine and stool exam, Liver function test, to exclude the diseases which are similar to VL. After that I did formol gel test, in addition to bone marrow aspiration and fine needle aspiration of spleen in all cases to detect the amastigotes Leishmania donovan bodies in the smear (glass slides) seen by haematologist after staining with leishman stain for diagnosis of VL.

**From table 1:** we found (low haemoglobin level, total WBC, and platelets count) in some cases, when compared to the normal blood count values in table 2, due to the immune system affected, and hypersplenism,<sup>4,12,13</sup> in addition to 8 cases with bronchopneumonia by chest x- ray (40%).

**From table 2:** we found formol gel test was positive in 10 cases (50%), bone marrow aspiration was positive in 12 cases (60%), while splenic aspiration was positive in all cases (100%) by using the Kenyan workers<sup>14,15</sup> method, it is easy and less painful technique to all patients.<sup>16</sup>

Both splenic and bone marrow aspirations showed the amastigotes of L. donovan bodies by haematologist, the splenic aspiration is very sensitive test (100%), more accurate method and easy to be performed if compared with other procedures,<sup>17</sup> after correction of anaemia and thrombocytopenia by fresh blood transfusion, prior to do splenic aspiration.

**From table 3:** we can detect the sensitivity test as follow:

Sensitivity test =  $\frac{\text{true positive}}{\text{true positive} + \text{false negative}} \times 100$

So the sensitivity rate for splenic aspiration =  $\frac{20}{20+0} \times 100 = 100\%$ , and the sensitivity rate

for bone marrow aspiration =  $\frac{12}{12+8} = 60\%$ , and the sensitivity rate for formol gel test

=  $\frac{10}{10+10} \times 100 = 50\%$ .

From this study we found that the splenic aspiration is very sensitive test for the diagnosis of VL and it is used in many countries like Kenya, this is regarded as primary diagnostic procedure, sometimes is done in the outpatient department without prior investigations.<sup>14,15</sup>

In other countries bone marrow aspiration or other method like IFAT are used to diagnose VL.<sup>17</sup>

In Sudan: lymph node enlargement is a prominent manifestation of the disease, they would provide a useful alternative site of diagnostic aspiration.<sup>18</sup>

Regarding to blood group as in table 4: I found that blood group A was more than other groups in 8 patients (40%), while blood group B in 6 patients (30%) then blood group O in 4 patients (20%), then blood group AB in 2 patients (10%), while in other studies, we found that the maximum percentage was in blood group (O), and minimum percentage in blood group (AB), and they found that the blood group was not a risk factor in the occurrence of VL, and the ABO – Rh blood groups were not associated with the occurrence of VL in Iranian patients<sup>25</sup>.

Blood transfusion was given after cross matching to those patients with anaemia and thrombocytopenia according to the level of haemoglobin and platelets count, prior to perform needle aspiration of spleen.

I observed all these cases for 24 hours after doing the procedure in paediatric emergency unit for any complications on the safe side by monitoring of vital signs, no complications were reported among all cases.

All cases were treated with sodium stibo gluconate (pentostam) in a dose of 15 mg/kgm single dose daily (iv) injection slowly for 3 weeks,<sup>9</sup> in addition to broad spectrum antibiotics for secondary bacterial infection, and antipyretic like paracetamol syrup and cold sponges to reduce fever.

From this study, I found that all cases were responded dramatically to pentostam therapy, firstly fever subsided then the appetite and mood of the patients were improved, while hepatosplenomegaly and lymphadenopathy were gradually decreased in size until disappeared completely by 2-3 months when I followed up all these cases in paediatric consultation outpatients department, without repeating bone marrow or splenic aspirations, only I did complete blood count for assessment of anaemia.

### **V. Conclusions:**

Visceral Leishmaniasis was found as a common public health problem in Yemen, mostly in rural areas because of rivers and swages in addition to dogs and poultry farms, this allowed to maximum density of Sand flies which are the vectors for transmission of the disease to human.

From this study we found that, fine needle aspiration of spleen for diagnosis of visceral Leishmaniasis (Kala-azar) is very sensitive test (100%), more accurate method and easy to be performed than other procedures, provided that the coagulation studies and platelets count are normal, if there is defect, fresh blood transfusion should be given to correct it, prior to do splenic aspiration.

Now days we can do this procedure under the abdominal ultra sound guidance.

Pancytopenia can occur due to infiltration of bone marrow by the parasites and hypersplenism causing anaemia, and infection and even purpuric rash with or without bleeding tendency, also nutritional anaemia like iron deficiency anaemia may occur because of anorexia as in those patients (80%).

Also from this study, we found that, the relationship of (VL) patients with blood group (ABO system) was not specific and not a risk factor in the occurrence of VL disease.

Using of insecticidal agents, destruction of dead animals, medico social education and using of fine window nets and taking the advices of veterinary doctors for the people who are breeding dogs, chickens and for slaughters and treatment of affected cases with pentostam therapy, playing an important role for reducing the morbidity and mortality rate of this disease, the early the diagnosis and treatment the best the prognosis (prophylaxis is better than treatment).





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