

Validation of Kala-azar diagnostic tests in a pediatric teaching hospital in Baghdad

Muhi K. Al-Janabi
Nadia A. Nasir
Ammar A. Hameed

MBCbB, FICMS, DCH, MRCPCH.
MBCbB, FABCM.
MBCbB.

Summary:

Background: Kala-azar is an important parasitic disease that affects children of all age groups with fatal outcome if left without treatment.

Objectives: Children admitted with Kala-azar were studied for evaluation of Indirect Immunofluorescent Antibody Test and Bone Marrow examination validity.

Patients and methods: A cross-sectional study was conducted on 60 children with Kala-azar admitted to Children Welfare Teaching Hospital - Medical City - Baghdad, for the period from the 1st of January 2011 to the 1st of February 2012. Information was obtained for each child including age, sex, residence, signs and symptoms, laboratory investigations and diagnosis was established by serology and/or bone marrow examination. Treatment and outcome were also noted.

Results: The majority (94%) of studied children were less than 5 years age group. Males were more than females with a ratio of 1.63:1. Sixty percent of patients were from Diyala governorate and 23% from Baghdad. The main clinical features were prolonged fever and hepatosplenomegaly (100%). Bone marrow examination was positive in 45% of suspected Kala-azar cases. IFAT was positive in 47(78%). IFAT sensitivity was 70% and specificity 15%. All cases were treated with Pentostam (sodium stibogluconate). No resistance to Pentostam treatment was detected. The case mortality rate was 2%.

Conclusion: The highest sensitivity in laboratory diagnosis among studied children was that obtained with a combination of bone marrow aspirate direct examination and IFAT. Conventional methods for diagnosis of visceral Leishmaniasis are still indispensable.

Keywords: Validity, kala-azar, diagnosis, Baghdad.

J Fac Med Baghdad
2014; Vol.56, No .3
Received April.2014
Accepted July. 2014

Introduction:

Kala-azar (visceral leishmaniasis) is an important public health problem, causes disease in children of all ages and it is fatal if not treated. It is caused by intracellular parasite which is transmitted by female vector sand flies. In most endemic area it is thought to be a zoonotic disease in which humans are only incidentally infected. Dogs and others carnivores are the most common reservoirs. Leishmania species are found in all countries except Australia. In Iraq it is caused mainly by *L. donovani* and to a lesser extent by *L. infantum*. An increase in the incidence of kala -azar worldwide in last years due to increase in sand fly density resulting from a reduction in malaria vector control programs and increase in vector and / or reservoir population. However mortality rate is declining because of improved case management in recent years due to availability of better diagnostic tools as r-K39 kits & oral drug miltefosine. (1, 2, 3, 4,5) Because some forms of Leishmaniasis heal spontaneously while others are chronic and progressive, immunoregulation of acquired host defense has been extensively investigated. (6)

Patients and Methods:

A cross-sectional study was conducted on 60 patients with visceral leishmaniasis who were admitted to Children Welfare Teaching Hospital - Medical City - Baghdad, for the period from the 1st January 2011 to the 1st February 2012. Information obtained was age, sex, residence, date of admission and discharge, clinical presentation and physical examination notes. The investigations which were done included complete blood picture, blood film, and liver function tests SGOT,SGPT, prothrombin time (PT) and partial thromboplastin time(PTT). The diagnosis was based on the result of bone marrow examination by looking for L.D bodies (amastigotes), and/or by IFAT test which was done by collecting few blood drops by a pin-prick onto Whatman No.3 filter paper, titer of ≥ 16 was considered positive. Bone marrow smear examination was considered the gold standard in the diagnosis of Kala-azar in this study. (7) Clinically suspected cases were those with negative BM smear and negative IFAT test and had responded to treatment for Kala-azar. Specific drug treatment (All studied patients were treated with sodium stibogluconate (Pentostam) given in a dose of ≥ 20 mg/kg/ day IM for 28 days. No other antileishmanial drugs was used.), supportive therapy and outcome of cases were noted. Statistical analysis was done by using statistical package for social sciences (SPSS) software for windows version 20. All data of different variables were

* Dept.of Pediatrics/ College of medicine /University of Baghdad
** Dept. of Community Medicine /College of medicine /University of Baghdad
*** Children Welfare Teaching Hospital /Medical City, Baghdad
Email:Muhi-janabi@yahoo.com

entered and analyzed with appropriate statistical tests (Chi Square, Fisher exact and T- tests). Graphpad instat Version 3.0 was used to calculate the sensitivity and specificity of the tests and to find the +ve and –ve predictive values. P.value \leq 0.05 was considered as significant.

Results:

Out of 60 children admitted with Kala-azar; 37(62%) were males and 23 (38%) were females with a male: female ratio of 1.63:1. The age range was 4 months - 12 years. The main age group affected was between 1 -2 year and they were 25 (42%). Those below 1 year of age were 16 (27%) and below 5 years were 56 (94%). Regarding residence; 36 (60%) of patients were from Diyala, 14(23%) from Baghdad, 4(6%) from Al-Kut, 2(3%) from Al-Diwanya, 1(2%) from each of Al-Najaf, Anbar, Babylon and Thi-Qar as seen in table-1.

Table-1: Characteristics of the studied children with Kala –azar

| Characteristics | | No. | % |
|-----------------|-----------------|-----|----|
| Age | <1 year | 16 | 27 |
| | 1-2 years | 25 | 42 |
| | >2years-5 years | 15 | 25 |
| | >5 years | 4 | 6 |
| Sex | Males | 37 | 62 |
| | Females | 23 | 38 |
| Residence | Dyala | 36 | 60 |
| | Baghdad | 14 | 23 |
| | Kut | 4 | 6 |
| | Others | 6 | 11 |

All studied children presented with fever and hepatosplenomegaly. The duration of illness before hospitalization was less than 1 month in 40 (67%) patients and more than 1 month in 20 (33%). Higher frequency of jaundice (40%) was noted in those with more than 1 month history of symptoms than those who were diagnosed within 1 month where Jaundice was noted in 5% of them. Severe anemia (Hb < 7 gm/dl) was recorded in 35 (58%) patients. Moderate anemia

with (Hb level between 7-9 gm/dl) was found in 15 (25%) of patients. Mild anemia with (Hb level >9 gm/dl) was found in 10 (17%) of patients. Leukopenia with (WBC count < 4000 cells/mm³) was found in 34(57%) of patients. Thrombocytopenia with (platelets count < 150 000 cells/ mm³) was found in 44 (73%) of patients. Blood film was normochromic normocytic in 40(67%) patients, while hypochromic microcytic in 20(33%). Ten (18%) patients had increased serum level of total serum bilirubin (TSB), 20(33%) with increased SGPT and SGOT, 8(13%) with decreased serum albumin, 6(10%) with decreased Total serum protein (TSP), 20(33%) with prolonged PT and PTT as seen in table-2.

Table-2: Hematological laboratory results in children with kala-azar

| Investigation | Results | No. | % |
|------------------------------|------------|-----|----|
| Haemolobin gm/dl | < 7 | 35 | 58 |
| | 7-9 | 15 | 25 |
| | >9 | 10 | 17 |
| White Blood Cells count /cmm | <4000 | 34 | 57 |
| | >4000 | 26 | 43 |
| Platelets /cmm | <150000 | 44 | 73 |
| | >150000 | 16 | 27 |
| Total Serum protein gm/dl | 6 | 6 | 10 |
| | \geq 6 | 54 | 90 |
| Total Serum bilirubin mg/dl | >1.2 | 10 | 18 |
| | \leq 1.2 | 50 | 82 |
| PT & PTT | Prolonged | 20 | 33 |
| | Normal | 40 | 76 |

In 27 (45%) patients, the diagnosis depended on clinical features and identification of parasite on bone marrow smear, 19 (32%) patients of them had IFAT positive. In 28 (47%) patients, the diagnosis depended on clinical features with positive IFAT but they had BM negative results. Direct bone marrow aspirate smear examination and IFAT were positive in 74% of patients. In 5(8%) patients; both bone marrow aspirate smear examination and IFAT were negative, and they responded to treatment as seen in table -3.

Table -3: The validity of IFAT test in the diagnosis of Kala –azar in comparison with bone marrow smear.

| | BM+ve | BM-ve | Total | Sensitivity (95% CI) | Specificity (95% CI) |
|---------|----------|----------|-----------|-------------------------|------------------------|
| IFAT+ve | 19 (32%) | 28 (47%) | 47 (78%) | 0.70 (0.50 to 0.86) | 0.15 (0.05 to 0.32) |
| IFAT-ve | 8 (13%) | 5 (8%) | 13 (22%) | | |
| Total | 27 (45%) | 33 (55%) | 60 (100%) | | |

P Value= 0.22; Positive Predictive Value = 0.40; Negative Predictive Value = 0.39

Fifty eight patients (97%) received antibiotics for bacterial infections (pneumonia and gastroenteritis). Thirty one patients (52%) received blood transfusion. Twelve patients (20%) received plasma transfusion. Ten (17%) patients received vitamin K. Fifty four patients (90%) discharged well after the

fever subsided .Two patients (3%) died because of septicemia, hepatic failure and bleeding tendency, those patients were presented with jaundice before treatment. Four patients (7%) were discharged on their parents> request with unknown outcome.

Discussion:

Majority of cases (94%) were under 5 years of age and the most frequent age group was between 1-2 years (42%). This agrees with other studies done in Iraq (8, 9, 10,11) and Iran (12). Male predominance among children with kala –azar has already been reported by many other studies; AL -Omary et al (10) and Mohammed et al (11). Male: female ratios were (1.27:1; 1.7:1) respectively, as compared to 1.63:1 reported in this study. The middle parts of Iraq were the most endemic areas and this agrees with other studies done in Iraq. (8, 9, 10,11) Eighty percent of patients were admitted to hospital during the period from December to April. Al-Abawdi et al (9) reported that most cases were admitted from November to April. Soleimanzade et al (12) in Iran reported most of cases admitted from January to April and both agree with this study. This might be attributed to the increment in sand flies activity and number occurring more during Summer and Autumn taking in consideration that the incubation period of parasite which is (2-8 months)(1) The main presenting clinical features were prolonged fever and hepatosplenomegaly in (100%) of studied patients and this agrees with other studies in Iraq. (8, 9, 10, 11) Anemia is a common finding in Kala-azar. In this study severe anemia (Hb <7 gm/dl) was found in 35(58%) patients and this level is slightly higher than other studies; Al-Saffar et al(13) (48%), Al-Abawdi et al (9) (41%), AL-Omary et al(10) (44%)and Mohammed et al (11) (49%). Pancytopenia is a known laboratory finding in Kala-azar; it is well shown in this study and agrees with other studies. (8, 9, 10, 11) In this study, blood film was mainly normochromic normocytic (67%), which agrees with Al-Saffar et al(13) (72.8%) and Mohammed et al(11) (62%), while in Al- Abawdi et al(9) study shown mainly hypochromic microcytic anemia (85.7%). Hypochromic microcytic anemia might be explained by possible iron deficiency anemia. In this study, sensitivity of IFAT was (70%), which was lower than that (90%) reported by Al-Saffar et al (13) (92%) reported by Al-Abawdi et al(9) (90%) reported by Mohammed et al (11) and (82%) reported by AL-Omary et al.(10) In a similar study done in Bangladesh (14), IFAT was found to be (100%) sensitive. In this study direct smear examination and IFAT were positive in 74% of patients. In Brazil; Yvone M. Et al. had reported direct smear examination and IFAT were positive in 80% of patients. (15) The specificity of IFAT test in this study was (15%) which disagrees with a study done in India (16) which showed IFAT specificity (70%), and other done in Bangladesh. (14)The less bone marrow positive cases (gold standard test) the more false positive IFAT which results in less specificity of IFAT. This might indicate the need for more proper search for L.D bodies in bone marrow smear examination. Regarding the duration of illness before diagnosis, it was found that those patient that had delay in diagnosis more than 1 month after onset of symptoms had higher frequency of jaundice (40%) than those diagnosed after short period of presentation (5%); this may be explained by the chronicity of illness and hepatocellular injury, and these results agrees with Mohammed study. (11) Pentostam was the drug of choice in the treatment of Kala-azar in Iraq, there was

good response to pentostam therapy together with supportive measures. The average stay in hospital for admitted cases was 6-7 days. This seems to be short and reflecting the limitation in numbers of beds in Children Welfare Teaching Hospital and the policy of discharging patients once the temperature drops to normal and by improvement in clinical picture to be followed-up as outpatients every 10-14 days for 1-2 months duration. Pentostam was given to all cases for 28 days. No clinical resistance to this drug recorded in this study, similar to Khlabus et al(17) in an Iraqi study ; in contrast to other countries such as India, East Africa or some parts of Latin America.(1) No relapse had been reported in the studied children.Two patients (3%) died in hospital which was less than that (9.36%) by Zijlstra et el in Kenya. (18) This is a recognized finding where the case fatalities were the least among other studies; this might reflect the high standard of care in Children Welfare Teaching Hospital in Medical City Complex in Baghdad in addition to no resistance to pentostam therapy.

Conclusions:

The highest sensitivity in laboratory diagnosis among studied children was that obtained with a combination of bone marrow aspirate direct examination and IFAT. Conventional methods for diagnosis of visceral leishmaniasis are still indispensable.

Author Contributions:

Muhi K. Al-Janabi: Study conception, Study design, and critical revision

Nadia A. Nasir : Acquisition of data analysis and interpretation of data

Ammar A. Hameed: Data collection, drafting manuscript

References:

1. Melby P.C. *Leshmaniasis in: Beherman R.E. Kleigman R.M., Jenson H.B., Editors, Nelson text book of pediatrics, 19th ed, saunders Elsevier, Philadelphia, 2011,Chap.277, Page 1186-1190.*
2. N.I. Neouimine. *Leishmaniasis in the Eastern Mediterranean region, Eastern Mediterranean heath. Journal World health organization, 1996; 2 (1):94-101.*
3. Postigo J.A. *Leishmaniasis in the World Health Organization in Eastern Mediterranean Region. International Journal of Antimicrobial Agents. 2010;36(1): 62-65*
4. Wittner M, Julia A., Ralph D. feigin et al. *Leishmaniasis Oski's pediatric: principle and practice, 4th ed, Philadelphia, 2006, Chap.220.page 1331.*
5. Gurumurthy Srividya, Arpita Kulshrestha, Ruchi Singh, Poonam Salotra. *Diagnosis of visceral leishmaniasis: developments over the last decade. Parasitology Research. 2012; 110(3):1065-1078.(IVSL)*
6. Wyler D.J. and Hamer D.H. *Leishmaniasis in Beherman R.E., Kleigman R.M., Arivin Pediatrics, 15th ed., Saunders, 1996, Chap. 244.6, Page 972-974.*
7. Chappuis F, Sundar S, Hailu A, et al. *Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol 2007; 5: 57–82.*

8. M.N. Naddawi, Maysem Al-Saffar and Jalil Al-Ezzi, Comparison of bone marrow examination and indirect immunofluorescent antibody test in the diagnosis of kala-azar. 2000; 42(4): 595.
9. Al-Abawdi, L.G., Kala -Azar (visceral leishmaniasis) in children, a thesis submitted to the scientific council of pediatrics in partial fulfillment for the degree of fellow ship of the Iraqi commission for medical specialization in pediatrics 1999.
10. Al-Omary S.M. Sensitivity of indirect immunofluorescent antibody test in diagnosis of Kala-Azar, a thesis submitted to the scientific council of pediatrics in partial fulfillment for the degree of fellow ship of the Iraqi commission for medical specialization in pediatrics 2001.
11. Mohamed D.J. Kala-Azar in Children with Special Emphasis on Those with Jaundice, a thesis submitted to the scientific council of pediatrics in partial fulfillment for the degree of fellow ship of the Iraqi commission for medical specialization in pediatrics 2010.
12. Soleimanzaden -G, Edrissian-GH, Movehhd-Danesh-AM, Epidemiological aspects of kala-Azar, Iran; Human infection, Bull-World-Health-Organ, 1993; 71(6): 759-62.
13. Al-Saffar ML. KA and value of bone marrow examination and IFAT in diagnosis, a thesis Submitted to the scientific council of pediatrics in partial fulfillment for the degree of fellow ship of the Iraqi commission for medical specialization in pediatrics 1998.
14. Muazzam-N, Rahman -KM, Asna-SM, Indirect fluorescent antibody test in the serodiagnosis of visceral leishmaniasis in Bangladesh, Bangladesh-Med-Res-Counc-Bull. 1992; 18(2): 77-81.
15. Yvone M. Brustoloni; Rivaldo V. Comparison of conventional methods for diagnosis of visceral leishmaniasis in children of the Center-West Region of Brazil. Braz J Infect Dis 2007; 11 (1) :106-109 (IVSL)
16. Sundar S, Rai M. Laboratory diagnosis of visceral leishmaniasis. Clin Diagn Lab Immunol 2002; 9: 951-958.
17. Khlabus Kh. Clinical and epidemiological features of kala-azar in Thi-Qar governorate. Medical Journal of Basrah University. 2007; 25(1):70-72.
18. Zijlstra-EE, Ali-MS, Kager-PA, Kala-azar a comparative study of parasitological methods and the direct agglutination test in diagnosis. Trans-R. Soc. trop-Med-Hyg. 1992 ; 86(5): 505-7.