Assessment of Clinical Features, Hematological Findings and Bone Marrow Status in Children with Kala azar Infection

Waleed Abdelazez Omar* PhD**, Raya Shakir Wadi**** BSc

ABSTRACT

Background: Visceral Leishmaniasis, also known as Kala azar, is a vector borne disease. The parasites spread from the inoculation site to the mononuclear phagocytic system involving the spleen, liver and bone marrow.

Objectives: Assessment of the clinical features, hematological findings and bone marrow status in children diagnosed as Kala azar by bone marrow examination.

Methods: This is a retrospective study conducted at the Hematology Laboratory of Children Welfare Teaching Hospital in Medical City, Baghdad, from October 2015 to February 2016. All the cases which had been diagnosed as visceral Leishmaniasis by bone marrow aspirates examination from January 2010 to December 2015 had included in the study.

Results: The study included 51 patients diagnosed as visceral Leishmaniasis by bone marrow aspirate examination; their mean of age was 37.8 months. Seven (14%) cases were acute lymphoblastic leukemia in remission. Twenty seven (53%) patients presented during winter season. The most common presenting features were: fever in 42 (82%) patients, splenomegaly in 42 (82%), hepatomegaly in 34(67%), pallor in 31 (61%). Laboratory investigation showed that all the patients (100%) presented with anemia, 50(98%) patients showed thrombocytopenia, 45(88%) patients showed leucopenia, 49(96%) patients showed neutropenia and 49(96%) patients presented with pancytopenia. Regarding the bone marrow aspirates, 41 (80%) aspirate showed normal marrow fragments, and the cellularity was normal in 38(95%) of those aspirates. The megakaryocytes were present in good number in 39(76%) aspirates. Erythropoiesis was active and of normoblastic maturation in 48(94%) aspirates; myelopoiesis was active with full spectrum of maturation in all (100%) aspirates.

Conclusion: Kala azar should be considered in child present with fever, splenomegaly, hepatomegaly and anemia or pancytopenia. There are no specific changes in the bone marrow aspirates apart from the presence of Leishman –Donovan bodies.

Keywords: Kala azar, Bone marrow aspirate, Visceral Leishmaniasis.

Iraqi Medical Journal Vol. 63, No. 1, January 2017; p.11-16.

Visceral Leishmaniasis (VL), also known as Kala azar, is a vector borne disease caused by a protozoan Leishmania Donovani. The female of a Phlebotomize Sand fly transmits the parasite from person to other or via animal reservoir[1]. Domestic dogs and foxes have been incriminated as reservoir[2]. Following an infected bite, parasites spread from the inoculation site to the mononuclear phagocytic system[3], involving the spleen, liver and bone marrow[2].

*Bone Marrow Transplantation Center, Medical city, Baghdad, Iraq.
**Medical Laboratory, Children Welfare Teaching Hospital, Medical City, Baghdad, Iraq.

VL has infrequently been reported from southern Europe, Northern Africa and Mediterranean according to the WHO[4].

The incubation period of the disease is generally 2-6 months; the onset can be sudden with high fever or gradual with intermittent fever. Diarrhea, joint pains, weight loss and bleeding gums occur in the acute phase; this is followed by progressive muscular wasting, protuberant abdomen, fever, anemia and hepatosplenomegaly[3].

The hematological abnormalities associated with the disease are normochromic normocytic anemia, pancytopenia, reticulocytopenia[3,5-7]. The ESR and CRP are elevated and

haemopgagocytosis has been observed in bone marrow specimen[2].

Laboratory diagnosis of the disease can be carried out using several serological tests including direct agglutination using freeze dried antigen[8], ELISAs using rk39 antigen[3], Indirect Immunofluorescent Antibody Test (IFAT)[9], and dipstick[10,11]. Immunochromotographic strip assay can, also, be used for the detection of Leishmania antibody[12].

Bone marrow or splenic aspirates may show the parasites, so called Leishman-Donovan bodies, in and around the macrophages[7], and in marrow biopsy[13]. The parasite is rarely seen in peripheral blood and then only in Buffy coat preparation[3]; diagnosis of VL can be, also, achieved by in vitro cultivation of bone marrow aspirate, which is more sensitive than microscopic examination of bone marrow aspirate, but this method is time consuming and expensive[14].

The DNA of Leishmania can be detected in the Gimsa stained bone marrow slides by polymerase chain reaction[15]; in recent years, the amplification of parasite DNA by PCR has proved to be rapid, sensitive and specific method for detection of Leishmania parasites in a number of different clinical material such as blood, bone marrow, lymph node and spleen[14].

The disease can be treated by pentostam (Sodium Stibogluconate)[16] or Liposomal Amphotericin B[2]. High mortality rate is expected if the disease is left untreated[14].

Aim of the study: Assessment for the clinical features, hematological findings and bone marrow status in children diagnosed as kala azar by bone marrow examination.

Methods

This is a retrospective descriptive study conducted in the Hematology Laboratory of Children Welfare Teaching Hospital in Medical City, Baghdad, in the period between October 2015 and February 2016. All the cases which were diagnosed as visceral leishmaniasis by bone marrow aspirates examination from January 2010 to December 2015 had been included in the study.

The provisional diagnosis of visceral leishmaniasis and the recording of the signs and symptoms in the patient's files were carried out by the physicians in Children Welfare Teaching Hospital.

The information which were recorded from the patients files included the following: code number of the aspirate, age and sex of the patients, date of patient presentation (month and year), clinical notes, and the results of full blood count which were performed by hematology analyzer (Ruby- Abbott - USA) and the results of examinations of the peripheral blood which was stained by Leishman stain.

The bone marrow aspirates were stained by Leishman stain and examined by experienced hematopathologists. The reports of bone marrow examination were studied in details and the information regarding the cellularity, megakaryocytes, meylopoiesis, erythropoiesis, granulocyte to erythrocytes ratio, lymphocytes percentage and the amount of Leishman-Donovan bodies in the bone marrow were recorded.

The reference range for hemoglobin (Hb), white blood cell (WBC) and platelet was according to Dacie and Lewis Practical Hematology[17] and the anemia considered when Hb is below 11 g/dl, leucopenia when WBC count below $5.0X10^9$/l and thrombocytopenia when platelet count below $150X10^9$/l

The data was presented by frequency distribution, and means and standard deviations were made for selected variables.

Results

The study included 51 patients diagnosed as visceral Leishmaniasis by bone marrow aspirate examination; their mean of age was 37.8 months (SD ±39.9) with range of 4 months to 12 years, but 41(80%) patients were below 5 years old age. The males were 33 (65%) patients

while the females were 18 (35%) patients and males to females ratio was 1.9:1.

Seven (14%) cases were acute lymphoblastic leukemia patients who were in remission.

Most of the cases of Kala-azar were presented during winter season; 27 (53%) patients presented during winter season, 11(22%) patients during summer, 8(15%) patients during spring and 5(10%) during autumn.

The most common presenting features were fever and splenomegaly; 42 (82%) patients suffer from fever for a duration ranged from 10 days to 150 days with mean of 34 days (SD ±34), while fever was not recorded in 9(18%) patients. Splenomegaly recorded, also, in 42(82%) patients, hepatomegaly in 34(67%) patients, pallor in 31 (61%) patients, abdominal distention in 7(14%) patients, lymphadenopathy in 3 (5%) patients, (Table 1).

Regarding the hematological laboratory findings, all patients (100%) presented with anemia (Hb below 11g/dl); 50 (98%) patients showed thrombocytopenia (platelet count below 150 X 10^9/l) at presentation and only one (2%) patient showed normal platelet count; 45 (88%) patients showed leucopenia (WBC count below 5.0 X10^9/l) and 6 (12%) patients showed normal WBC count but no one showed leukocytosis. The absolute count for neutrophils was below 1.5 X 10^9/l in 49 (96%) patients, (Table 2).

Forty nine (96%) patients presented with pancytopenia.

Table 1: Signs and symptom in Kala azar patients at presentation.

The sign	No. of patients	% of the sign
Fever	42	82
Splenomegaly	42	82
Hepatomegaly	34	67
Pallor	31	61
Abdominal distention	7	14
Lymphadenopathy	3	5
Lethargy,	2	4
Epistaxis	2	4
Vomiting, diarrhea, cough, cachexia, poor feeding, loss of weight, night sweating,	1 (for each sign or symptom)	2 (for each sign or symptom)

Table 2: Mean, standard deviation and range of each laboratory hematological parameter for all the patients (51) included in the study.

Parameter	Mean	±SD	Range
Hb(g/dl)	7.4	1.5	4.2-10.6
Platelet count x10^9/l	47	44	8-233
WBC count x10^9/l	3.1	1.7	0.7-8.5
Neutrophils count x10^9/l	0.9	0.4	0.2-2.3

SD: Standard Deviation

Regarding the bone marrow aspirate, 8342 marrow aspirate procedures were carried out in Children Welfare Teaching Hospital for the period from January 2010 to December 2015 and only 51(0.6%) aspirates yield LD bodies; of these 51 aspirates, 40 (78%) aspirates showed large number of Leishman-Donovan (LD) bodies and these LD bodies present extracellular

and intracellular while 11 (22%) aspirates show few number of LD bodies.

Forty one (80%) aspirate show marrow fragments and the cellularity were normal in 38(95%) aspirates. More over 2(5%) aspirates were hypercellular and showed marrow fragments while 10(20%) aspirates were diluted so that the cellularity cannot be assessed.

The megakaryocytes were active in all (100%) aspirates and present in good number in 39(76%) aspirates and present in few number in 13 (24%) aspirates; the erythropoiesis was active and of normoblastic maturation in 48(94%) aspirates while it was hyper plastic in 3(5%) aspirates; the myelopoiesis was active and with full spectrum of maturation in all (100%) aspirates and the average of granulocytes to erythroid elements (G/E) ratio was 2.2: 1. The average of lymphocytes percentage was 20% with range of 4-40% of all nucleated marrow cells. One (2%) aspirate showed haemophagocytosis.

There were no excess blasts in the marrow of the seven leukemic patients e.g. the leukemia was in remission and the diagnosis of Kala-azar was carried out after completion the treatment.

Discussion

There are many studies and researches on visceral Leishmaniasis and each study concentrate or concern with one or more aspects of the disease including clinical features, methods of diagnosis, outcome of treatments and epidemiology; this study was restricted to cases diagnosed by bone marrow aspirate.

Visceral Leishmaniasis (Kala-azar) is transmitted by female vector sand flies (Hermes)[16], but according Al-Kaby et al 2007, 75% of mothers in rural areas in Baghdad do not know that Hermes transmit the disease[18] and this will affect the protection from the infection.

The study showed that the mean of age for the patients was 37.8 months, and 80% of patients were below 5 years age, more over the males were more than females; ratio 1.9:1. These results were in agreement with the reports of WHO[19]; and were comparable with the study of Al-Shamsi et al 2008 which stated that 97% of the patients were under 5 years old age[20].

The study showed that most of the cases presented in Winter season and such result was similar to the study of Al-Hamash 2012 which was carried out in Baghdad and Al-Muhammadi et al 2004 which was carried out in Babel governorate[1,9], but differ from the study of Al-Ani et al 2012 which was carried out in Al-Anbar governorate which showed the highest incidence was in the spring season[16]. This seasonal variation is related to the sand fly season that last from April to November and to the relatively long incubation period of the parasite.

The study showed that the most common presenting signs were fever and splenomegaly, followed by hepatomegaly and pallor and those results were similar to those reported by the WHO[19] and other studies; so that Al-Muhammadi et al 2004, Al-Hamash 2012 and Al-Ani 2012 had reported that the most common signs were fever, splenomegaly, hepatomegaly and pallor[1,9,16].

This study showed that the duration of fever ranged from 10 days to 5 months while Al-Shamsi et al 2008 study reported fever from 2 weeks to 3 months[20].

The study included 7 cases of ALL in which Kala-azar was diagnosed and this is expected because these patients are immunocompromised due to anti leukemic cytotoxic drugs; Drexler and Hasbro 2014 stated that visceral Leishmania is a well known opportunistic infection in sever immunodeficiency , particularly in patients living in area where leishmaniasis is endemic[21]. The important question is how the patient gets the infection; is it through sand fly or through blood and blood product transfusion or activation of pre-existing infection.

The study showed that all patients showed anemia (Hb below 11 g/dl) and this

similar to the results of Al-Shamsi et al 2008[20].

This study showed that 96% of patients present with pancytopenia; Al-Awadi et al, a study which included children and adults in 2009 found that kala-azar is the third cause of pancytopenia after acute leukemia and aplastic anemia in 74 patients presented with pancytopenia in Babel, Iraq , but the study included children and adults[22].

This study showed that 0.6% of all marrow aspirate procedures had yield LD bodies which differ from the study of Musa et al 2014 which showed 1%[23]. However, we may propose that this study is more representative because the study of Musa had included the marrow aspirates in Children Welfare Teaching Hospital for only 6 months throughout 2010 while the current study included aspirates for the last 6 years in the same hospital.

The study showed that 80% of marrow aspirates were cellular and 5% were hypercellular and this agree with Cartwright et al 1948[24], in addition to that the megakaryiopoiesis, myelopoiesis and erythropoiesis were active so the peripheral pancytopenia can be explained, partly by the presence of splenomegaly resulting in hypersplenism is responsible for the peripheral pancytopenia.

The study showed that most of the aspirates showed active myelopoiesis in spite of the infection of the reticuloendothelial cells of bone marrow and this can be explained under the study of Cotterell et al 2000 who found that infection of the stromal macrophages will increase their capacity to support myelopoiesis in vitro, an effect mediated mainly through the induction of granulocyte macrophage-colony stimulating factor and Tumor Necrosis Factor –alfa[25].

The present study showed that the erythroid maturation was normoblastic and the myeloid /erythroid ratio shifted from the normal of approximately 3.5:1 to an average of 2.2:1. This findings agreed with Cartwright et al 1948[24].

The bone marrow of one patient showed hemophagocytosis and this agree with Matnani and Ganapathi 2016 who stated that acquired hemophagoytic lymphohisteocytosis associated with many disorders including Leishmaniasis[26].

Conclusion; Kala azar should be considered in child presented with fever, splenomegaly, hepatomegaly and anemia or pancytopenia. The bone marrow is usually cellular and marrow elements still active and no specific changes occur apart from the presence of Leishman –Donovan bodies, speculating that the peripheral cytopenia may be due to hypersplenism.

Acknowledgement: the authors thank Dr. Najiha Ahmed Ameen (FIBMS Hematopathology) and Dr. Wisam Majeed Abed (FIBMS Hematopathology) and Mr. Basil Hatam for their permission and assistance in performing this study.

References

1- Al-Hamash S M. Study of Visceral Leishmaniasisin children of Iraq. Mustansiriya Medical Journal 2012; 11(2):15-19.
2- Goldman L, Ausiello D. Cecil Medicine, 23rd ed. 2007-Saunders Elsevier, Philadelphia.
3- Hoffbrand AV, Catovsky D, Tuddenham E, Green A. Postgraduate Haematology, 6th ed. Blackwell publishing, UK. 2011, p. 967-968
4- Hellal M, Kundu S. Visceral Leishmaniasis involving the bone marrow. Blood. 2013; 122(5): 627.
5- Bain BJ. Dyserythropoisis in visceral leishmaniasis. Am J Hemat 2010; 85(10):781.
6- Hoffbrand AV, Moss PAH. Essential Haematology, 6th ed. Wiley Blackwell publishing, UK 2011 p:346.
7- Arceci RO, Hann IM, Smith OP. Pediatric Hematology, 3rd ed. Blackwell Publishing, USA 2006 p:763.
8- Jacquet D, Boelaert M, Seaman J, et al. Comparative evaluation of freeze dried and liquid antigens in the direct agglutination test for serodiagnosis of visceral leishmaniasis. Tropical Medicine and International Health 2006;11 (12):1777-84.
9- Al-Muhammadi M, Al-Shujiri GS, Noor M. Hematological changes in children suffering from visceral leishmaniasis. Medical Journal of Babylon 2004; 1(3): 325-330.
10- Tarish HA. Hematological and epidemiological study of visceral leishmaniasis in pediatric patients in Mid-Euphrates area. Alkadisiah Medical Journal.2010; 6 (10): 118-130.
11- Tarish HR, Jawad FM, Dawood A. Dipstick strip test versus bone marrow test for diagnosis of visceral leishmaniasis in pediatric patients in Mid-Euphrates area. The Medical Journal of Basrah 2009; 27(2) :71-76.

12- Al-Timmimi MF, Guirges SY, Al-Tae AA. Evaluation and comparison of immunochromatography and immunoflurescent techniques in diagnosis and epidemiological studied in Iraqi Kala-azar. Iraqi Postgraduate Medical Journal 2009; 8 (3):285-90.
13- Bibas M, Nonno FD. Leishman-donovan bodies in the bone marrow biopsy. Blood 2011; 118(1):8.
14- Al-Nhhas S, Shaaban M, Hammoud L. Contribution study of visceral leishmaniasis in Syria. Saudi Medical Journal 2005; 26(5):490-2.
15- AL-Mishry MK, Ali SK, Al-Shimary MN, Mahdi NK. Diagnosis of visceral leishmaniasis by conventional PCR to DNA extracted from Giemsas solution-stained slides. Basrah Journal of Science 2014; 32: 75-85.
16- Al-Ani ZR, Al-Hamwandi AM, Al-Maaeeni AA, Al-Taaie MK. Kala azar in Al-Anbar governorate, western Iraq. Anbar Medical Journal 2012;10(1):41-9.
17- Bain BJ, Bates I, Laffan MA, Lewis SM. Dacie and Lewis Practical Hematology. 11th ed. Elsevier Churchill Livingstone, China 2011, p.:11-21
18- Al-Kaby FA, Al-Shimary FA, Al-Maliky MK. Predisposing factors for leishmaniasis in rural areas in Baghdad. Al-Taqani 2007; 20(1):79-84.
19- WHO-Control of Leishmaniases. Report of a meeting of WHO expert committee on control of leishmaniases, Geneva, 22-26 March 2010.
20- Al-Shamsi M, Mohsin RK, Abdullah AH. Kala azar in Qadisiah: A clinicoepidimiological descriptive study. J Fac Med Baghdad 2008;50(2):203-9.
21- Drexler B, Holbro A. Unexpected bone marrow finding in a patient with pancytopenia after hemopoietic stem cell transplantation. Blood 2014;124 (5):678.
22- Al-Awadi NB, Al-Awad AS, Al-Yasiri HH. Patterns of pancytopenia according to the cause In Babylon. Medical Journal of Babylon 2009; 6 (3, 4):434-40.
23- Musa A, Abed WM, Al-Saeed RM, Al-Hadad S. Bone marrow procedures in Children Welfare Teaching Hospital, Medical City Complex indication and results. J of Med Baghdad 2014; 56 (3):254-7.
24- Cartwright GE, Chung HL, Chang AN. Studies on the pancytopenia of Kala-azar. Blood.1948; 3 (3): 249-75.
25- Otterell SEJ, Engwerda CR, Kaye PM. Leishmania donovani infection of bone marrow stromal macrophages selectively enhance myelopoisis by amechanism involving GM-CSF and TNF alfa. Blood 2000; 95(5):1640-50.
26- Matnani M, Ganapathi KA. Hemophagocytic lymphohistiocytosis associated with visceral leishmaniasis. Blood 2016;127(4).

IMJ 2017;63(1):11-16.