Evaluation the Levels of IFN-Gamma , IL-10 and Concentration of Zn in Children with Visceral Leishmaniasis

Mais N. Kamil *, Sabah Al-Najar ** , Nahla Ghanim ***

ABSTRACT:

BACKGROUND:

Visceral Leishmaniasis (VL) is a systemic infection of the reticulo- endothelial system that could affect on immune system and biochemical parameters like the concentration of trace elements which may be significantly changed .

OBJECTIVE:

Evaluating the level of cytokines (INF- γ ,IL-10) and concentration of Zn in Visceral Leishmaniasis in children after diagnosis .

PATIENTS AND METHODS:

A total of (98) child their ages rang (6m-5y) were attending to the Central Public Health Laboratory and Teaching Laboratories of Medical City suspected to be infected with kala-azar, the diagnosis was done by both IFAT technique and Rapid Kala-azar (r-K39) detecting test and evaluating the level of cytokines (INF- γ, IL-10) by ELISA and concentration of trace element (Zn) by Atomic Absorption Technique .

RESULTS :

The dipstick test (r-K39) showed a high sensitivity of (92.1%) compared to IFAT (73.6%) with a specificity of 100% for both tests. Serum samples of 56 child with positive results in IFAT and rK39 test were used for the investigation of IL-10, IFN-γ. The mean levels of IL-10 (80.207±77.54 Pg/ml) and IFN- γ (5.426±4.599 IU/ml) were highly significant increased in patients compared to healthy controls .The mean level of serum Zn (69.71±7.97µg/dl) was significantly low in VL patients compared to healthy controls .

CONCLUSION :

This study showed that the (r-K39) dipstick test could be more sensitive than IFAT technique in the diagnosis of VL with a specificity of 100 % for both test according to clinical diagnosis. Both IFN-γ & IL-10 were significantly increased in VL patients as compared to controls group. The concentration of Zinc was significantly lower in VL patients than healthy controls.

KEYWORD : visceral leishmaniasis , IFN-gamma , IL-10, trace element (Zn).

INTRODUCTION:

Visceral leishmaniasis is a systemic infection of the reticulo-endothelial system [(1)]. It is commonly known as kala-azar (black fever), caused by *Leishmania donovani* & *Leishmania infantum* in the old world and *Leishmania chagasi* in new world [(2)]. Visceral Leishmaniasis is endemic in 62 countries, with a total of 200 million people at risk , an estimation 500.000 new cases each year worldwide and (41000) recorded deaths in the year 2000 [(3)].The disease usually detected in infants and children, and according to that VL is considered to be infantile type [(4)]. Female sand fly *Phlebotomus* become infected by biting infected animals (e.g. rodent or dogs) or human [(5)]. The clinical symptoms are characterized by prolonged and irregular fever associated with chills and rigor ,splenomegaly,hepatomegaly and pancytopenia [(6)] . Risk factor for development of clinical disease include malnutrition, immune suppressive drugs & HIV co-infections [(7)]. The outcome of the clinical form of the disease is critically influenced by the immune response developed by the host [(8)].

*DNA Department, Medico Legal Institute, Ministry of Heath.

**Microbiology Department, College of Medicine ,Baghdad Universit.y.

***Pathology and Immunology, Department Teaching Laboratories, Medical City, Ministry of Heath.

A systemic infection with the spread of the parasite to liver, spleen and other organs ,is accompanied by high titer of circulating anitbodies and depression of Th1 T-cell mediated immunity with decreasing production of IFN-γ &IL-12 and marked upregulation of IL-4 & IL-10 [9]. IFN-γ is an important Th-1 cytokine crucial for the control of intracellular infections , so it needed for the control and protection of leishmania infection[10]. Some studies on immune response in VL have showed increased the levels of serum IFN-γ and other cytokines such as (IL-1,IL-6&IL-10) [11]. Recent reports have indicated that despite the presence of high levels of IFN-gamma ,infected host may fail to induced intracellular signaling mechanisms [10].Experimental evidences indicate that IL-10 plays an important regulatory role in the progression of the VL, also it has a suppressive ability on IFN-gamma mediated microbicidal activity of macrophages that established for other disease also [12].The diagnosis of VL is difficult it established either by serological tests such as IFAT, dipstick(r-K39) test, ELISA, direct agglutination test (DAT) or by bone marrow examination and spleen & liver biopsy [13] .

The major trace elements (micronutrient) play an important roles in biological systems by participation in the structures or as an active site of Metalloenzymes, an imbalance of mineral levels either by excess or deficiency cause alterations in respective serum level [14]. Many pathological conditions results in alteration of some trace elements such as bronchitis , pneumonia and some parasitic diseases like toxoplasmosis ,cutaneous leishmaniasis and recently in visceral leishmaniasis[15], Although there are many trials of nutritional intervention and their effect on infectious diseases. The occurrence of marginal and moderate deficiencies of trace element in human has served as the impetus to determine whether supplementation with these elements has the potential effect to prevent, attenuate, and treat infectious diseases [16].

MATERIALS AND METHODS :

This study was conducted in Central Public Health Laboratory and Teaching Laboratories of Medical City in a period between (Novamber 2007-April 2008).A total of (98) child their ages rang (6m-5y) were included in this study. These children were divided into the following groups :

Seventy six(76) children their ages range from 6m-5y divided into (42 male and 34 female) who were clinically diagnosed as VL suffering from prolong fever and hepatosplenomegaly and considered as patients group . 22 healthy child attending to Special Surgical Hospital at Medical City / Baghdad for Plastic Surgery and considered as healthy control group .All the (98) serum samples were diagnosed by IFAT (Anti Leishmanial Ab.Kits , Euroimun Germany) and rK39 dipstick (Rapid immunochromatogrphic strip assay test , InBios USA) for the detection of VL infection, only the samples who were positive by both IFAT & rK39 dipstick (truly infected with VL) were used to evaluate the serum cytokines level (IFN-γ , IL-10) by ELISA (Enzyme Immunoassay Kits ,Biosource Finland) and concentration of Zn by atomic absorption spectrophotometer compared to healthy controls. The separated serum samples were divided into (3) aliquots and were immediately frozen at -20˚c till tested.

Statistical Analysis:

The suitable statistical methods were used in order to analyze and assess the results, they include the followings:

Descriptive statistics:

Statistical tables including observed frequencies with their percentages.

Summary statistic of the readings distribution (mean, SD).

Graphical presentation by charts.

Inferential statistics : These were used to accept or reject the statistical hypotheses, they include the followings:

Chi-square(χ^2),

Student test (t-test).

Pearson Correlation (r).

Note: The comparison of significant (P-value) in any test were:

S= Significant difference (P<0.05).

HS= Highly Significant difference (P<0.01).

NS= Non Significant difference (P>0.05). (17)

RESULTS:

The distribution of visceral leishmaniasis (VL) among children according to their age and sex ,were shown in Fig.(1) highest percentage of +ve VL among male and female age groups (1-3) years, while the lowest percentage was among male & female age groups(3-5) years respectively. A highly significant differences prevalence (P < 0.01) in patients in contrast to controls group tested by IFAT and (r-K39) showed in table (1). Data in table (2,3) demonstrated the sensitivity of r-K39 dipstick and IFAT (92.1%, 73.6 %; respectively) and

specificity for both rK-39 dipstick and IFAT (100%) in contrast to clinical diagnosis. The value of mean ± SD levels of IFN-γ in patients group was significantly higher than that in controls group **(**5.426±4.599 IU/ml, 0.418±0.122 IU/ml ; respectively, P<0.01 **).** Also, the value of mean ±SD levels of IL-10 in patients group was significantly higher than that in controls group **(** 80.207±77.54 Pg/ml 19.173±16.241 Pg/ml; respectively, P<0.01 **)** . The values of mean ±SD of serum zinc was significantly lower than in controls group (69.71 ±7.97 µg/dl, 110.45±12.53 µg/dl; respectively, P<0.01**)** .The data in Table (5) demonstrated the relations between serum (Zn) and serum cytokines (IFN-γ, IL-10) , where a negative correlation between serum Zn & both (IFN- γ,IL-10) in VL patients **(** r = - 0.394 , r = - 0.30 **)** respectively, data on this table also showed a low positive correlation (r = 0.125) between (IFN-γ & IL-10) in patients with VL infection.

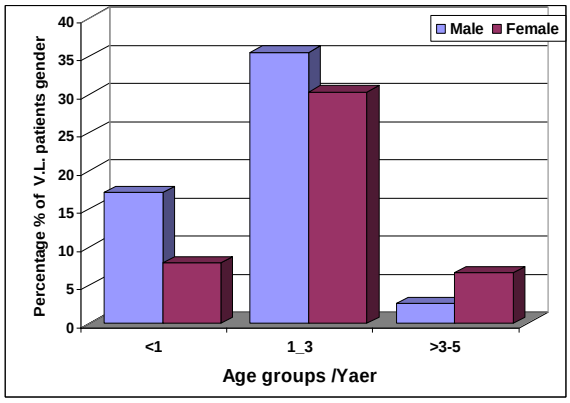

Figure 1: Distribution of VL Patients According to Sex & Age Group.

Table 1: Distribution of Studied Groups (Healthy Control & Visceral Leishmaniasis patients) According to IFAT & rK-39 dipstick test.

Parameters			Studied groups		Total	Comparison of significant	
		Control		V.L. patients		P-value	Sig.
		N	0	56	56		

IFAT	Positive	%	0	73.7	57.1	0.00	Highly Sig. (P<0.01)
	Negative	N	22	20	42		
		%	100	26.3	42.9		
Total		N	22	76	98		
		%	100	100	100		
Serum rK-39 Dipstick	Positive	N	0	70	70	0.00	Highly Sig. (P<0.01)
		%	0	92.1	71.4		
	Negative	N	22	6	28		
		%	100	7.9	28.6		
		N	22	76	98		
Total		%	100	100	100		

Table 2 : Validity of the Strip rK-39 tes[illegible]pared with clinical diagnosis of VL.

Validity		Clinical diagnosis		Total
		Positive	Negative	
Serum rK-39 dipstick	Positive	70	0	70
	Negative	6	22	28
Total		76	22	98
Sensitivity		92.105 %.		
Specificity		100 %.		
PPV.		100 %.		
NPV.		78.57 %.		
Accuracy		93.877 %.		

PPV.: positive predictive value
NPV.: negative predictive value

Table 3: Validity of the IFAT Compared with clinical diagnosis for VL.

Validity		Clinical diagnosis		Total
		Positive	Negative	
IFAT	Positive	56	0	56
	Negative	20	22	42
Total		76	22	98
Sensitivity		73.68 %		
Specificity		100 %		
PPV.		100 %		
NPV.		52.38 %		
Accuracy		79.59 %		

Table 4 : Mean level of IFN- γ (IU/ml) and IL-10 (Pg/ml) & serum Copper (µg/dl) in studied groups (Patients & Controls).

Parameters	Control N=22	Patients N=56	Comparison of Significant	
			P-value	Sig.
Mean ±SD IFN- γ (IU/ml)	0.418±0.122	5.426±4.599	0.00	

Mean ±SD IL-10 (Pg/ml)	19.173±16.241	80.207±77.54	0.002	Highly Sig. (P<0.01)
Mean ±SD S.Zinc (µg/dl)	110.45±12.53	69.71±7.97	0.00	

Table 5 : Correlation Between Concentration of Zinc & cytokines level among VL patients

IL-10 (Pg/ml)		IFN-γ (IU/ml)	Correlation
Serum Zinc (Mg/dl)	Pearson Correlation(r)	-0.394	-0.30
	P-value	0.00	0.007
	Sig.	HS	HS
IFN-Gamma (IU/ml)	Pearson Correlation(r)		0.125
	P-value		0.276
	Sig.		NS

DISCUSSION:

Figure (1) showed no significant differences between males and females patient with VL and this is in agreement with other study [18] and the highest VL prevalence were at age group (1-3) years and this may be due to the fact that children in this age group are susceptible to infection due to their development of immune system and their maternal immunity start to decrease [18,19]. Table(1) showed highly significant positive results for VL patients by IFAT compared to control groups where (73.7%) which agree with [20], these results could be due to circulating Abs may be not detected because of poor target antigens or because the disease is so active that antigens released into circulation have adsorbed all the antibodies, also in table (1) we demonstrated that (92.1%) of patients were positive by rK39 dipstick compared to control group ,which agreed with[21,22] which showed 90% positive results by rK39 test, [23] stated that 96% of suspected VL patients were positive by r-K39 dipstick test . The validity of r-K39 test compared to clinical diagnosis where 70 patient were positive by r-k39 and clinically diagnosis as showed in table (2) this give the test 92.1% sensitivity with 100% specificity which is agree with [1,22] , the IFAT test showed sensitivity of 73.6% and specificity of 100 % at table (3) compared to clinically diagnosis[24] these results, included ,circulating Abs may not be detected because of poor target antigens. Table (4) showed highly significantly increased in serum IFN-γ patients as compared to controls group , which agreed with the [10,19,25], table (4) also demonstrated that IL-10 was significantly higher in patients than in healthy controls which agreed with results from

es[19,25]. These results confirmed that high IL-10 level in combination with high parasite associated with persistence and severity of VL , its also one of the reason why children are more susceptible to Leishmaniasis [25]. The VL patients in table (4) showed significant decrease in serum zinc concentration of patients [15,26] these results might be due to VL infection where patients suffering from nausea , loss of appetite . Data in table (5) demonstrated a negative correlation between serum zinc and (IFN-γ,IL-10) was found in children with VL who showed a weak positive relation between (IFN-γ & IL-10) these results could be due to the number of selected patients in this study therefore a larger data is preferable, also late presentation of the Iraqi patients or most of them consult a doctor when the disease reach to acute or chronic stages where both IL-10 & IFN-γ acts at the same time .

CONCLUSION:

Throw this study we concluded that the higher prevalence of VL was among children at the age group (1-3) years without significant differences between male and female.

The rK39 dipstick test is more sensitive than IFAT test while both techniques showed specificity of 100% according to clinical diagnosis .

Both IFN-γ & IL-10 was significantly increased (P<0.01) in VL patients as compared to controls group.

The concentration of Zinc was significantly lower (P<0.01) in VL patients compared to healthy

controls .Negative correlations found between zinc concentration and IFN-γ and IL-10 levels (r =- 0.394 , r =- 0.30) respectively.

REFERENCES:

1. Purva M.,Jyotish S.& Kumar Chaudan: Evaluation of rapidimmunochromategraphic test for diagnosis of Kala-azar and Post kala azar Leishmaniasis at a tertiary care center of north India : Indian J.Med. Res. 2005;123:485-90 .
2. Samiran Saha , Smriti Mondal, Antara Banerjee, Jayeeta Ghose, Sudipta Bhowmick & Nahid Ali: Immune responses in kala-azar. Indian .J Med. Res. 2006;123:245-66 .
3. WHO. The world health report 2001. Geneva
4. Annual Report Rajendra Memorial research Institute of Medical Sciences: The leishmaniasis spp. 2002:40 .
5. Bhattacharya S.K., Dipika Sur & Juntra Karbwang : Childhood visceral leishmaniasis ,Indian J. Med. Res. 2006;123: 353-56.
6. Berman JD. : Human leishmaniasis: clinical, diagnostic, and Chemotherapeutic developments in the last 10 years. Clin. Infect.Dis.1997; 24:684-703.
7. Alvar J, Canavate C, Gutierrez-Solar B, Jimenez M, Laguna F, Lopez-Velez R, etal. Leishmania and human immunodeficiency virus co-infection: The first 10 years. Clin. Microbiol. Rev. 1997; 10:298-319.
8. Peruhype-Magalha˜es y V., Martins-Filho O. A., Prataz A. , de A. Silvaz L.,Rabello A., Teixeira-Carvalho A. , Figueiredo R. M., S. F. Guimara˜es-Carvalho, T.C. A. Ferrariyy & R. Correa-Oliveira : Immune Response In Human Visceral Leishmaniasis: Analysis of the Correlation Between Innate Immunity Cytokine Profile and Disease Outcome. Scandinavian Journal of Immunology 2005;62;487–95.
9. Bacellar O, Brodskyn C, Guerreiro J et al.: Interleukin 12 restores IFN-γ production and cytotoxic responses in visceral leishmaniasis. J.Infect. Dis. 1996;173:1515–18.
10. Asrat H., Tom Van Der Poll, Nega B.& PIET A. K.: Elevated Plasma Levels of (IFN-γ,IFN-γ Inducing Cytokins, and IFN-γ Inducible CXC Chemokines in Visceral Leishmaniasis Am. J. Trop. Med. Hyg., 2004;71;561–67.
11. de Medeiros I.M. ,Castelo A.& Salomao R.: presence of circulating levels of IFN-gamma ,IL-10 and TNF-alpha in patients with VL. Rev.Int. Med. Trop.Sao Paulo.1998;40:31-34.
12. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. : Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J. Exp.Med. 1991;174:1209-20.
13. Pearson R.D.,Cox G.,Jeronimo, S.M.B. Castracane J.et al :Visceral Leishmaniasis a model for infection –induced cachexia . Am. J. Trop.Med.Hyg.1992;1:47:8-15.
14. Huges S. & Kelly P. : Interactions of malnutrition and immune impairment, with specific reference to immunity against parasites *Parasite Immunology*, 2006;28:577–88.
15. Johan V.W., Gisélia S., Argemiro D'Oliveira Jr., Anibal F Santos Jr, Carlos H.Costa, Edgar M Carvalho, Aldina Barral & Manoel Barral-Netto: Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: an ex vivo and in vitro study.BMC Infectious Diseases, 2004; 4:50 doi:10.1186/1471-2334-4-50.
16. Mark L. Failla : Trace Elements and Host Defense: Recent Advances and continuing Challenges , J.N. 2003:1443-46.
17. Sorlie D.E : Medical biostatistics & epidemiology : Examination & board review First ed. Norwalk, Connecticut, Appleton & Lange , 1995:47-88.
18. Olga Zerpa, Marian Ulrich, Margarita Benitez, Concepción Ávila, Vestalia Rodríguez, Marta Centeno, Doris Belizario, Steven G Reed, Jacinto Convit :Epidemiological and Immunological Aspects of Human Visceral Leishmaniasis on Margarita Island, Venezuela . Mem Inst Oswaldo Cruz ,Rio de Janeiro, 2002; 97:1079-83 .
19. Aseel S.Mahdi : study of some immunological parameters for children infected with VL A thesis submitted to colleagd of health and medical technology for a degree of Master science 2007.
20. Antonio Cascio, Lucio di Martino, Paolo Occorsio, Raffaella Giacchino4, Salvatore

Catania , Anna Rita Gigliotti, Camilla Aiassa, Chiara Iaria, Salvatore Giordano,Claudia Colomba, Valentina Frasca Polara, Lucina Titone, Luigi Gradoni,Marina Gramiccia & Spinello Antinori : A 6 day course of liposomal amphotericin B in the treatment of infantile visceral leishmaniasis: the Italian experience. Journal of Antimicrobial Chemotherapy .DOI: 10.1093/jac/dkh279 Advance Access publication ,2004;54:217–20.

21. Bern, C., S. N. Jha, A. B. Joshi, G. D. Thakur & M. B. Bista : Use of the recombinant k39 dipstick test and the direct agglutination test in a setting endemic for visceral leishmaniasis in Nepal. Am. J. Trop. Med. Hyg. 2000;63:153–57.

22. Silvio F. Guimara˜ es Carvalho, Elenice M.L., Ralph C.,and Reynaldo D. :Performance of recombinant K39 antigen in the diagnosis of Brazilian visceral leishmaniasis. Am. J. Trop. Med. Hyg.2003; 68: 321–24.

23. Israel Cruz, Carmen Chicharro, Javier Nieto, Begon˜a Bailo, Carmen Can˜avate,Marı´a-Concepcio´n Figueras & Jorge Alvar :Comparison of New Diagnostic Tools for Management of Pediatric 'ooMediterranean Visceral Leishmaniasis. Journal of clinical Microbiology , 2006:2343–234.

24. Fattaneh M. ,Mahdi F., Bahador S., Mohammad H.M.,Gholamreza H. :Comparison of Serological Methods (ELISA, DAT and IFA) for Diagnosis of Visceral Leishmaniasis Utilizing an Endemic Strain .Iran.J.Immunol. 2007;4.

25. Nasim A.,Sumita S. & Poonam S. : Elevated levels of interferon-gamma, interlukine-10 and interlukine-6 during active disease in Indian kala-azar. Clin. Immunol. 2006;119:339-45.

26. Bern C., Rashidul H., Rajib Ch. M. Ali, Kati M.K., Louise V., Josef A., M. A. Wahed, Yukiko W., Robert F. B., Johan W.,W. Evane Secor & James H Magurie : The Epidemiology Of Visceral Leishmaniasis and Asymptomatic Leishmanial Infection in a Highly Endemic Bangaladeshi Village Am.J.Trop. Med. Hyg. 2007;76: 909–14.