Evaluation of the levels of IFN-gamma, IL-10 and Copper in children with Visceral Leishmaniasis

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Summary:

Background: Visceral Leishmaniasis(VL) is a systemic infection of the reticulo- endothelial system that could affect the immune system and biochemical parameters like the concentration of Copper which may be significantly hanged .

Objective: The study aims to evaluate the level of cytokines (INF- γ ,IL-10) and trace element (Cu) in Visceral Leishmaniasis in children after diagnosis .

patients and Methods: A total of 98 children, whose their ages ranged (6 menthes -5 years) were attending the Central Public Health Laboratory and Teaching Laboratories of Medical City, who were suspected to be infected with kala- azar, who were diagnosed by both IFAT technique and Rapid Kala-azar (r-K39) detecting test and trying to evaluate the level of cytokines (INF- γ , IL-10) by ELISA and concentration of (Cu) 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 Cu (171.54±10.37 µg/dl) in VL patients.

Conclusions: 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. Copper concentration was significantly higher in VL patients than healthy controls.

Keyword: Visceral Leishmaniasis , IFN-gamma , IL-10, trace element (Cu).

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Introduction :

Visceral leishmaniasis (VL) 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). 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 type 1 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

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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 elements 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).

Patients 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) children, their ages ranged (6m-5y), were included in this study. These children were divided into the following groups : Seventy six children their ages range from 6 m-5 y divided into (42 male and 34 female) who were clinically diagnosed as VL suffering from prolonged fever and hepatosplenomegaly and considered as patients group . Twenty two healthy children attending to Special Surgical Hospital at Medical City / Baghdad for Plastic Surgery and considered as healthy control group .All the ninety eight serum samples were diagnosed by IFAT and rK39 dipstick test 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 and concentration of Cu 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) and 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) and 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 of (3-5) years. 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 levels of serum copper in patients group were significantly higher than that of controls group **(**171.54 ±10.37 µg/dl, 141.82±12.40 µg/dl ; respectively P<0.01 **)** table (4) . The data in table (5) demonstrated the relations between concentration of serum (Cu) and serum cytokines (IFN-**γ**, IL-10) , where a positive correlations between serum copper & IFN-γ, IL-10 (r = 0.322 , r = 0.278) respectively , data on this table also showed a low positive correlation (r = 0.125) between (IFN-γ & IL-10) in patients with VL infection.

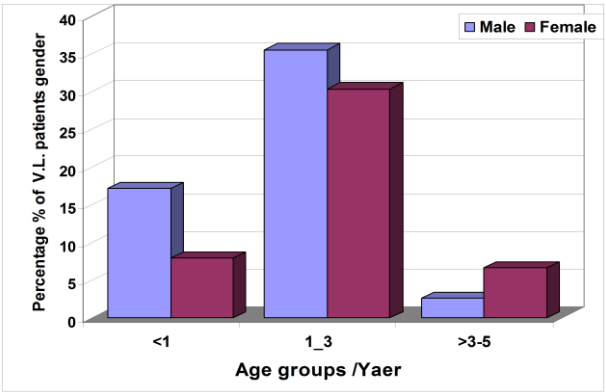

Figure (1):Distribution of VL patients according to sex & age group

Table (1) Distribution of studied groups (Healthy Control &Visceral Leishmaniasis patients) according to IFAT & rK-39 dipstick test

Comparison of significant		Total	Studied groups			Parameters	
Sig.	P-value		V.L. patients		Control		
Highly Sig. (P<0.01)	0.00	56	56	0	N	Positive	IFAT
		57.1	73.7	0	%		
		42	20	22	N	Negative	
		42.9	26.3	100	%		
		98	76	22	N	Total	
		100	100	100	%		
Highly Sig. (P<0.01)	0.00	70	70	0	N	Positive	Serum rK-39 Dipstick
		71.4	92.1	0	%		
		28	6	22	N	Negative	
		28.6	7.9	100	%		
		98	76	22	N	Total	
		100	100	100	%		

Table (2) Validity of the Strip rK-39 test as compared with clinical diagnosis of VL

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

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

Table (3) Validity of the IFAT compared with clinical diagnosis for VL

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

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

Comparison of Significant		Patients N=56	Control N=22	Parameters
Sig.	P-value			
Highly Sig. (P<0.01)	0.00	5.426±4.599	0.418±0.122	Mean ±SD IFN- γ (IU/ml)
	0.002	80.207±77.54	19.173±16.241	Mean ±SD IL-10 (Pg/ml)
	0.00	171.54±10.37	141.82±12.40	Mean ±SD S.Copper (µg/dl)

Table (5) Correlation between concentration of Copper & cytokines level among VL patients

IL-10 (Pg/ml)	IFN-γ (IU/ml)	Correlation	
0.278	0.322	Pearson Correlation(r)	Serum Copper (Mg/dl)
0.014	0.004	P-value	
S	HS	Sig.	
0.125		Pearson Correlation(r)	IFN-Gamma (IU/ml)
0.276		P-value	
NS		Sig.	

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 Antonio Cascio ,*etal* (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 Bern, C., *etal* and Silvio F., *etal* (21)(22) which showed 90% positive results by rK39 test. Israel Cruz , *etal* (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 agreed with Purva M.,*etal* and Silvio F., *etal* (1)(22) . The IFAT test showed sensitivity of 73.6 % and specificity of 100 % at table (3) compared to clinically diagnosis Fattaneh M. *etal* (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 Asrat H., *etal* , Aseel S.Mahdi and Nasim A.,*etal* (10)(19)(25) , table (4) also demonstrated that IL-10 was significantly higher in patients than in healthy controls which agreed with results from studies Aseel S.Mahdi and Nasim A.,*etal* (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 a highly significant serum copper concentration compared to controls group which agreed with Johan V.W., *etal* and Banerjee M., *etal* (15)(26) this could be related to environmental or genetic factors. A positive correlation between serum copper and (IFN-γ,IL-10) was found in children with VL who showed a weak positive relation between (IFN-γ & IL-10) table (5) . 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 .

Conclusions:

we concluded that the higher prevalence of VL was among children at the age group (1-3) years without significant differences between males and females .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 . Copper concentration was significantly higher ($P<0.01$) in VL patients compared to controls group. Copper concentration initiate a positive correlations with (IL-10 , IFN-γ) in VL patients ($r = 0.322$) ($r = 0.278$) respectively.

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