ISSN: 0067-2904
GIF: 0.851

Study of Some Serological Tests on Patients with Visceral Leishmaniasis

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Abstract

This study aimed to measure the alterations in serum zinc (Zn) and acute phase proteins (C-reactive protein and Ceruloplasmin) concentrations in patients with visceral leishmaniasis (VL). A total of 62 individuals were enrolled in this study : 52 individuals were infected with visceral leishmaniasis and 10 individuals as healthy control. Serum zinc levels were significantly ($p<0.05$) decreased in patient group(76.25 ± 4.59 µg/dl) when compared with healthy control (103.75 ± 3.77 µg/dl) . C-reactive protein , as a mediator of innate immunity, removed damaged cells by activating the classical complement pathway revealed elevated levels in patients (4.36± 0.23mg/l) when compared with the healthy control (2.50 ± 0.28mg/l). The level of Ceruloplasmin was also significantly ($p<0.05$) increased in VL patients (0.82±0.05 mg/dl) as compred with healthy subjects (0.43±0.03mg/dl). These results showed that serum essential trace elements Zn, C-reactive protein and ceruloplasmin concentrations have been changed in VL patients. This may be a part of defense strategies of organism.

Keywords :Visceral leishmaniasis, Zinc ,CRP, Ceruloplasmin

دراسة بعض الاختبارات المصلية على مرضى اللشمانيا الاحشائية

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الخلاصة :

الهدف من الدراسة الحالية هو قياس التغيرات الحاصلة في تركيز كل من الزنك والسي رياكتف بروتين والسيريلوبلازمين .شملت الدراسة ٦٢ شخص ،٥٢ منهم مصاب باللشمانيا االحشوية و ١٠ أشخاص أصحاء. أظهرت النتائج ان تركيز الزنك في المرضى المصابين (76.25 ± 4.59 µg/dl) انخفض بصورة معنوية بالمقارنة مع الأشخاص الأصحاء (103.75±3.77µg/dl). السي رياكتف بروتين الذي يعمل على إزالة الخلايا المحطمة عن طريق تنشيط المسار الاعتيادي للمتمم أظهرت النتائج ارتفاع معنوي($P<0.05$) لمستوى هذا البروتين في المرضى المصابين (4.36± 0.23mg/l) بالمقارنة مع الأشخاص الأصحاء (2.50 ± 0.28 mg/l). أظهرت النتائج أيضا ارتفاع مستوى السريلوبلازمين في الأشخاص المصابين (0.82±0.05 mg/dl) بالمقارنة مع الأصحاء (0.43±0.03 mg/dl) عند مستوى معنوية ($P<0.05$). بصورة عامة وضحت هذة الدراسة حصول تغير في تركيز كل من الزنك و السي رياكتف بروتين والسيريلوبلازمين في المرضى المصابين باللشمانيا وهذا التغير يمكن أن يعتبر كوسيلة دفاعية من قبل الشخص المصاب.

Introduction:

Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania* and spread by the bite of certain types of sand flies. The disease can present in three main ways as: cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis [1].Visceral leishmaniasis (VL) is one of the World's most neglected and poverty-related diseases, affecting the poorest people

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in developing countries associated with malnutrition, weakness of the immune system, displacement, poor housing, illiteracy, gender discrimination, and lack of resources [2].VL is found throughout parts of the old and new worlds and can infect humans as well as domestic and wild animals [3]. Transmission occurs in 88 tropical and sub tropical countries where the sand fly vector is present [4] 500,000 cases of VL occurs each year, more than 90% of the VL cases reported from 5 countries : India, Bangladesh, Brazil, Nepal and Sudan and soon Iraq with 4,000-5,000 annual cases will be added as 6th country to the above list [5].

Trace elements are needed for many metabolic and physiological processes in the human body [6]. One of the mechanisms, the serum redistribution of essential trace elements Zn, Cu and Fe together with the increase in synthesis of acute-phase proteins (like ceruloplasmin), which takes place during the course of most infections, is well established [7] . The changes are part of defense strategies of organism and are induced by the hormone like substances interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) [8,9].

The purpose of the present study was to investigate the status of essential trace element zinc concentration and acute phase proteins ceruloplasmin and c-reactive protein levels in serum of VL patients.

Materials and Methods

Subjects

This study included fifty –two patients diagnosed to be having visceral leishmaniasis collected from the Central teaching hospital of Pediatric in Baghdad. There ages ranged from 5 months to 2 years during the period December 2012 to March 2013.

Blood Sample Collection

Five ml of venous blood were collected from each patient. For serum collection, the tube was centrifuged for 10 minutes at 3000 rmp, then dispensed into a sterile eppendorf tube and stored at – 20 until use.

Evaluation of Zinc, CRP and Ceruloplasmin levels

Zinc concentration was evaluated by colorimetric determination way according to the manufacture instructions of (Via Milano, Bussero (Milan) Italy) by using of 50 µl of serum

10 µl was obtained from sera of patients to determine the levels of CRP according to the manufacture instructions of (DRG,USA).

Ceroluplasmin concentration was obtained by taking 5 µl from sera of patients according to the manufacture instructions of (Siemens Healthcare Diagnostics Products GmbH,Germany).

Statistical analysis

The data were expressed as mean ± SE student T-test and ANOVA table was used to examine the probability current study samples groups.IBM and SPSS computer programs version 21 was used to analyze the data. P value of less than 0.05 was considered as statistically significant.

Results and Discussion

All 52 studied children proved having visceral leishmaniasis, their ages ranged between 5 months to 2 years and the infection rate was significantly higher ($p<0.01$) in age more than one year 32 (61.53%), 21(40.38%) of them were males and 31(59.61%) were females, the percentage of females was significantly higher ($p<0.01$) than males as shown in Table-1.

Table 1-The percentage distribution of VL in studied children according to age and sex

		No.of cases(%)	Total No.(%)	Chi-square Value
Age	Less than one year	20(38.46)	52(100)	9.514 **
	More than one year	32(61.53)		
Sex	male	21(40.38)	52(100)	7.277 **
	Female	31(59.61)		

Serum zinc concentration was significantly ($p<0.05$) decreased in VL patients (76.25± 4.59 µg/dl) than in control group (103.75± 3.77 µg/dl) Table- 2. This fact was confirmed by other studies, [10-12] who also showed that the level of serum zinc was decreased in visceral leishmaniasis patients. Zinc is required as a cofactor for the function of intracellular enzymes that may be involved in protein, lipid and glucose metabolism. Zinc may participate as integral component of several antioxidant enzymes [11].

The decreased serum zinc levels in patients with leishmaniasis are probably due to the redistribution of zinc from plasma to the liver. Cytokines (IL-1) released during the acute-phase response of the host's immune system activates the synthesis of metallothionein in the liver and other tissues; metallothionein participates in the process of energy production and protection against reactive oxygen species that may be generated during the infection, and it is a metal-binding protein which appears to alter the hepatic uptake of zinc [13].

On the other hand, patients with VL revealed a significant ($p < 0.05$) increase in the levels of CRP (4.36± 0.23 mg/l) as compared with the control group (2.50 ± 0.28mg/l) Table-2, the present result was agreed with previous results that showed CRP level increased in VL patients such as [14,10]. CRP is an acute phase protein, which is produced principally by hepatocytes [15]. It is an annular (ring-shaped), pentameric protein found in the blood plasma, its level as a response to inflammation. Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system [16].

This protein increased significantly after most forms of tissue injuries and infections as a non-specific innate defense mechanism of the host. It is a marker of inflammatory reactions and cytokine activation [17].

The wide distribution of phosphocholine in polysaccharides of pathogens and in cellular membranes enables CRP to recognize a range of pathogenic targets as well as membranes of damaged and necrotic host cells [18].

Results also showed that the levels of ceruloplasmin were significantly ($p < 0.05$) higher in VL patients (0.82±0.05mg/dl) as compared with control group (0.43±0.03mg/dl). This result was in agreement with previous results of [19] who revealed increasing of ceruloplasmin level in dogs infected with VL. This fact also agreed with those obtained by [20] who they found that ceruloplasmin levels were increased in VL infections.

Ceruloplasmin is a blue plasma glycoprotein that is synthesized primarily in hepatocytes, It is the major copper-carrying protein in the blood which carries about 70% of the total copper in human plasma while albumin carries about 15% [21].

Table 2- The levels of zinc, CRP and ceruloplasmin in VL patients and control group.

Groups	Treatment	Mean ±SE	Probability
Patients	Zinc	76.25±4.59 µg/dl	P<0.05
Control		103.75± 3.77 µg/dl	
Patients	CRP	4.36±023 mg/l	P<0.05
Control		2.50±0.28 mg/l	
Patients	Ceruloplasmin	0.82±0.05 mg/dl	P<0.05
Control		0.43±0.03mg/dl	

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