Tikrit Journal of Pharmaceutical Sciences 2007, 3 (2):112 - 116

Measurement of CD4 and CD8 in Cutaneous Leishmanisis Patients and their Relation with Zn Deficiency

*Hussein S. Aswed AL-Obaidi - **Ashoor R. Sarhat - ***Abd A. Salman - ****Azad Kamal
*Dept. of Microbiology / College of Medicine / Tikrit University
**Dept. of Pediatrics / College of Medicine / Tikrit University
***Dept. of Community Medicine / College of Medicine / Tikrit University
***Al – Hawija General Hospital

Received 14 / 6 / 2007 – Accepted 15 / 7 / 2007

ABSTRACT:-

Cutaneous leishmainasis still is a common skin infection worldwide and because it's type and of clinical course depends on patient's immune system which in turn affected by zinc. To assess the relation of serum zinc, $CD4^+$, and $CD8^+$ with the disease. A total of 107 patients with CL were studied during period between October/2004-April/2005 in the dermatology department of Al-Haweja general hospital. Patients were divided in to two groups according to immune system development and Zn requirement. Sixty healthy individuals were used as control group, and divided into two groups, first groups 20 individuals' ages ranging from 1-15 years and second groups above 15 years their ages ranging from 16 to 60 years and sub-divided into 20 males and 20 females. After history and examination confimation of diagnosis made by skin smears. Serum zinc level and $CD4^+$ and $CD8^+$ were done for all patients and control. The mean of serum Zn concentration in patients above 15 years of age was significantly low 7.61 ± 0.03 µmol / l for male, and 6.69± 0.31 for female in comparison to 14.08 ± 0.04 µmol/l for male, and 12.02 ± 0.02 for female in similar control group. The mean percent of $CD4^+$ in patients above 15 years of age was significantly low 36.5 ± 0.98% for male, and 36.29 ± 0.06 % for female in comparison to 59.95 ± 0.16%% for male, and 58.9±0.14 % for female in similar control group. The mean percent of $CD8^+$ in patients above 15 years of age was significantly low 22.37±0.05% for male, and 22.47±0.04% for female in comparison to 30.95±0.12% for male, and 31.15±0.15% for female in similar control group. The $CD4^+/CD8^+$ ratio mean in patients above 15 years of age was significantly low 1.63±0.004 for male, and 1.6±0.004 for female in comparison to 1.94±0.01 for male, and 1.89±0.01for female in similar control group. All patients with CL have zinc deficiency, and have decreased in $CD4^+$, $CD8^+$, and decreased $CD4^+/CD8^+$ T lymphocytes.

Keywords: CD4 and CD8 in cutaneous leishmanisis, Zn deficiency in cutaneous leishmanisis.

قياس الخلايا التائية $CD4^+$, $CD8^+$ في الليشمانيا الجلدية وعلاقتها مع الخارصين

حسين ساهر اسود العبيدي – عاشور رفعت – عبد احمد سلمان - آزاد كمال

المستخلص:-

الليشمانيا الجلدية لا تزال من الأمراض الشائعة حول العالم ولان نوع والمسار السريري للمرض يعتمد على الجهاز المناعي والذي يتأثر بدوره بالخارصين. ولتقييم علاقة مستوى الخارصين والخلايا التائية $CD4^+$, $CD8^+$ مع المرض. تم دراسة 107 مريضا باللشمانيا الجلدية خلال الفترة بين تشرين الاول2004—نيسان2005 في قسم الأمراض الجلدية في مستشفى الحويجة . المرضى تم تقسيمهم إلى مجموعتين تبعا لنمو الجهاز المناعي ومتطلبات الخارصين. وشملت الدراسة ستين شخصا معافى كمجموعة سيطرة وتم تقسيم المرضى ومجموعة السيطرة إلى مجموعتين: المجموعة الأولى وتضم عشرين شخصا تتراوح أعمارهم بين 1-15 سنة والمجموعة الثانية تضم أربعين شخصا (عشرين أنثى وعشرين ذكرا). بعد التاريخ المرضي والفحص السريري تم تأكيد التشخيص بلطخة الجلد. تم فحص مستوى الخارصين المصلي، $CD4^+$ و $CD8^+$ لمجموعتي المرضى والسيطرة. معدل تركيز الخارصين المصلي في المرضى فوق عمر 15 سنة كان منخفضا انخفاضا معنويا 7.61 ±0.03 مايكرومول / لتر للذكور، 6.69 ± مايكرومول / لتر 10.03 للإناث بالمقارنة مع 14.08 ±0.04 مايكرومول / لتر للذكور، 12.02 ± 0.02 مايكرومول / لتر للإناث لمجموعة السيطرة المتشابهة. وكانت معدل نسبة $CD4^+$ في المرضى فوق عمر 15 سنة كان منخفضا انخفاضا معنويا 36.5 % ± 0.098 للذكور، 36.29% ± 0.06 للإناث بالمقارنة مع 59.95 % ± 0.16 للذكور، 58.9 % ± 0.14 للإناث لمجموعة السيطرة المتشابهة. وكانت معدل نسبة $CD8^+$ في المرضى فوق عمر 15 سنة كان منخفضا انخفاضا معنويا 22.37% ± 0.05 للذكور، 22.47% ± 0.04 للإناث بالمقارنة مع 30.95% ± 0.12 للذكور، 31.15

% ± 0.15 للإناث لمجموعة السيطرة المتشابهة. وكان معدل نسبة $CD4^+/CD8^+$ في المرضى فوق عمر 15 سنة كان منخفضا انخفاضا معنويا 22.37% ± 0.05 للذكور، 22.47% ± 0.04 للإناث بالمقارنة مع 30.95 % ± 0.12للذكور، 31.15 % ± 0.15للإناث لمجموعة السيطرة المتشابهة. كل المرضى المصابين بالليشمانيا الجلدية كان عندهم نقص الخارصين وكان عندهم انخفاضا $CD4^+/CD8$ ومعدل $CD4^+$ و $CD8^+$.

الكلمات الدليلية: $CD8^+$, $CD4^+$ في الليشمانيا الجلدية، نقص الخارصين في الليشمانيا الجلدية.

INTRODUCTION:-

Cutaneous Leishmaniasis (CL) is traditionally divided into: Old World (Mediterranean Basin, Africa, India, China, Soviet Union, and Asia Minor) and New World (primarily Central and South America, excluding Chile and Uruguay). While the New World CL is caused by the L. brasiliensis and L. mexicana. The cutaneous leishmaniasis of old world is due to L. tropica, L. major, L. *aetheopica*, L.*infantum*, and L.*donovani*. The two species that present in Iraq are: L. tropica, agent of anthroponotic cutaneous leishmaniasis, and L. major, agent of zoonotic cutaneous leishmaniasis and both occur in Iraq [1]. As far as the resulting patterns of illness arise from the tissue tropism of the leishmanial species and the host's immune response, principally the cell mediated component of immunity [2]. Because the immunity, cell mediated immune, T lymphocytes and macrophages and IgM of host play an important role in elimination of intracellular parasites [3]. The importance of zinc for the immune system is clear as zinc deficiency causes lymphopenia and reduced immune capacity among affected humans and also causes a 50% reduction in leucocytes and 40–70% reduction in antibody-mediated and cell-mediated immunity [4].

MATERIALS AND METHODS:-

A total of 107 patients (57% of patients were male and 43% were female) with CL were included in this study during the period between October-2004 to April-2005 in the dermatology department of Al Haweja general hospital in AL-Haweja district. Patients were divided in to two groups according to development of immune system and due to difference in dietary zinc requirement for maturation and growth for all age groups. Sixty healthy individuals were used as control group and divided in to two groups; the first groups contain 20 individuals their ages ranging between 1-15 years, and the second groups contain individuals their ages ranging from 16 to 60 years and sub-divided into 20 males and 20 females. After careful examination and history taken, lesional skin smears were done for detection of intracellular amastigotes. Blood sample was collected from each patients and control groups, was transferred immediately into 2 tubes as follows: one for lymphocytes separation technique to detect $CD4^+$, and$CD8^+$ by trypan blue exclusion test was done to assess cell viable analysis of peripheral blood T. lymphocytes $CD4^+$, $CD8^+$ subset based on immunostaining technique. Ficoll 40 (Pharmacia fine chemicals) was used for isolation T-lymphocytes. , and the other for detection of serum zinc concentration, atomic absorption/flame emission spectrophotometer model (shimadzu A.A.6200) fitted with air-acetylene flame was used for measurement of zinc concentration.

RESULTS:-

In the present study, the mean of serum Zn concentration in patients above 15 years of age was significantly low 7.61 ± 0.03 µmol / l for male, and 6.69± 0.31 for female in comparison to 14.08 ± 0.04 µmol/l for male, and 12.02 ± 0.02 for female in similar control group. Serum Zn concentration in patients under 15 years of age significantly low 5.57 ± 0.02 in comparison to 9.6 ± 0.07 in similar control groups shown in table 1. In the present study, the mean percent of $CD4^+$ in peripheral blood lymphocytes in patients above 15 years of age was significantly low 36.5 ± 0.98% for male, and 36.29 ± 0.06 % for female in comparison to 59.95 ± 0.16%% for male, and 58.9±0.14 % for female in similar control group. The mean percent of $CD4^+$ in patients under 15 years of age significantly low 16.5±0.03%, in comparison to 44.2±0.13% in similar control groups shown in table 2. In the present study, the mean percent of $CD8^+$ in patients above 15 years of age was significantly low 22.37±0.05% for male, and 22.47±0.04% for female in comparison to 30.95±0.12% for

male, and 31.15±0.15% for female in similar control group. The mean percent of $CD8^+$ in patients under 15 years of age significantly low 16.5±0.03%, in comparison to 29.5±0.13% in similar control group as shown in table 2. The $CD4^+/CD8^+$ ratio mean in patients above 15 years of age was significantly low 1.63±0.004 for male, and 1.6±0.004 for female in comparison to 1.94±0.01 for male, and 1.89±0.01for female in similar control group as shown in table 2.

DISCUSSIONS:-

The clinical outcome of infection thus not only depends on the species involved, but also on the patient's immunocompetence. In recent years, a protective immune response against intracellular pathogens, such as *Leishmania, Listeria* and mycobacteria, has been defined as type 1 (Th1), whereas protection against extracellular pathogens, such as helminths, requires a type 2 (Th2) response. The process of elimination of intracellular pathogens, such as *Leishmania*, requires a Th1 type immune response, whereas a dominant Th2 response leads to exacerbated disease. Experimental human zinc deficiency decreases Th1 but not Th2 immune response. This may be explain what this study revealed that serum Zn concentration in all CL patients is significantly decreased and goes in agreement with what found by [5] that decreased serum zinc in Turkish LCL patients infected by *L. major*. CL patients might be already having Zn deficiency therefore was infected by CL disease because Zn deficient people are more susceptible to infectious diseases [6]. This finding is in agreement with that found by Weyenbergh et al[7] they found that Zn concentrations of CL patient was significantly decreased, even that its in agreement with what found by Kocyigit et al. they found that low serum Zn and Iron levels in serum of CL patients in Turkey[8]. The T cell-mediated immune response is extremely important to define the outcome of the disease; however, the underlying mechanisms involved are not fully understood [9]. The significantly low mean percent of $CD4^+$ in peripheral blood lymphocytes in patients and in both male and female in comparison to that of similar control group. This finding is in agreement with that found by Fraker PJ, *et al.* [10], who found that the peripheral lymphoid organs, T-lymphocytes were progressively depleted from the spleen, lymph nodes and peripheral blood in Zn-deficient animals, and is in agreement with results of Fernandez G, *et al.* [11], in that the activity and the number of T-lymphocytes were decreased in Zn deficiency child, further more that is in agreement with those of Ruhl, *et al.* [12], in that the process of blast transformation and the number of T-lymphocytes in peripheral blood decreased in Zn deficiency human and is in agreement with that found by Dardenne *et al.* [13] they found for thymulin hormone Zn is an essential cofactor for differentiation and maturation of $CD4^+$ and $CD8^+$ T-lymphocytes and activity of this process decreased due to Zn deficiency. This could be explained that the decreased in mean of $CD4^+$% might be related to Zn deficiency and inability of CL patients were included in our study to eliminate the infection. The significantly low mean percent of $CD8^+$ in peripheral blood lymphocytes in patients and in both male and female in comparison to that of similar control group and this is in agreement with that found by Coto *et al.* [14] who found that Zn deficiency has effect on proliferation of $CD8^+$ T-lymphocytes, and is in agreement with that found by Crea *et al.* [15], that Zn deficiency is associated with decreased T cell proliferation after mitogen stimulation, and is in agreement with Wellinghausen *et al.* , significance of Zn for leukocytes biology, furthermore is in agreement with Shi *et al.* that Zn deficiency affected the phenotypic distribution of spleenic T–lymphocytes cells bearing $CD3^+$, $CD4^+$, $CD8^+$[16,17]. The significantly low $CD4^+/CD8^+$ ratio mean in patients above 15 years of age for male, and female in comparison to that of similar control group and this might be related to conformational change in their thymulin hormone and decreased in activity of thymulin hormone due to Zn deficiency therefore their immune power were decreased and they were infected by CL. This finding is in agreement with that found by Cung *et al.* [18], who found that Zn is bound to thymulin hormone in a 1:1 stiochiometry structure and thymulin activity, in *vitro* and *vivo* in both

animals and humans is dependent on plasma Zn concentration and thyulimn hormone responsible to keep the ratio of $CD4^+/CD8^+$ in normal range, and is in agreement with that found by Raqib R, *et al.* [19], that $CD4^+/CD8^+$ ratio decreased due to Zn deficiency in child infected with shigellosis. In contrast no significant difference was found between the mean of ratio $CD4^+/CD8^+$ in patient's ≤ 15 years group and control group since. This could be explained that this age group their immunity is not developed and their thymulin hormone was not active due to Zn deficiency.

CONCLUSIONS:-

All patients with CL have zinc deficiency, and have decreased in $CD4^+$, $CD8^+$, and decreased $CD4^+/CD8^+$ T lymphocytes.

Table (1) Serum Zn Concentration in Patients and Control According to Age and Sex

Serum Zn Concentration in Above 15 Years Old			
	Patients µmol/l	Control µmol/l	
Male	7.61 ± 0.03	14.08 ± 0.04	(P< 0.05)
Female	6.69± 0.31	12.02 ± 0.02	(P< 0.05)
Serum Zn Concentration in Under 15 Years Old			
	5.57 ± 0.02	9.6 ± 0.07	(P< 0.05)

Table (2) $CD4^+$ and $CD8^+$ Mean Percent in Patients and Control According to Age and Sex

$CD\ 4^+$ Mean in Above 15 Years Old			
	Patients	Control	
Male	36.5 ± 0.98%	59.95 ± 0.16%%	(P< 0.05)
Female	36.29 ± 0.06 %	58.9±0.14 %	(P< 0.05)
$CD\ 4^+$ Mean in Under 15 Years Old			
	16.5±0.03%,	44.2±0.13%	(P< 0.05)
$CD8^+$ Mean in Above 15 Years Old			
Male	22.37±0.05%	30.95±0.12%	(P< 0.05)
Female	22.47±0.04%	31.15±0.15%	(P< 0.05)
$CD8^+$Mean in Under 15 Years Old			
	16.5±0.03%	29.5±0.13%	(P< 0.05)
$CD4^+/CD8^+$ Mean in Above 15 Years Old			
Male	1.63±0.004	1.94±0.01	(P< 0.05)
Female	1.6±0.004	1.89±0.01	(P< 0.05)
$CD4^+/CD8^+$ Mean in Under 15 Years Old			
	1.44±0.003	1.49±0.01	(P> 0.05)

REFERENCES:-

1-Ashoor R. Sarhat. Combined cutaneous and visceral forms of leishmaniasis; case report. Tikrit journal of pharmaceutical sciences. 2006; 2(1):52-7.

2-Mebrahtu Y B, Lawyer P G, Hendricks L D, Muigai R, Oster C N, Perkins P V, Koech D K, Pamba H, and Roberts C R: Concurrent infection with Leishmania dono-vani and Leishmania major in a Kenyan patient: clinical description and parasite characterization. Am J Trop Med Hyg.1991;45:290–296. www.pubmed.gov.

3-Roitt I, Brostoff and J Male D. Immunology. 2000; 6th ed. PP: 66,199,156, 262, 316.Mosby. London.

4-F.T.Al-Gurairi, M.Al-Waiz and K.E.Sharquie: Oral zinc sulphate in the

treatment of recalcitrant viral warts: randomized placebo-controlled clinical trial. British Journal of Dermatology. 2002; 146 (3): 423.

5-Johan Van Weyenbergh et al. Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: an ex vivo and in vitro study. BMC Infectious Diseases 2004, 4:50

6-Lee BW, HK Yap, FT Chew, Quah K, Prabhakaran GS, Chan SC, *et al.* Age and sex related changes in lymphocyte subpopulations of healthy Asian subject: from birth to adulthood. Cytometry. 1996; 26: 8-15.

7-Weyenbergh VJ, Santana G, Argemiro G, JR D'Olivera, Costa HC, *et. al.* Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: An ex vivo and vitro study. BMS. Infectious diseases. 2004; 4: 50.

8-Kocigit A, Erel O, Gurel MS, Avci S and Akteje. Alteration of serum Selenium, Zn, Copper and Iron concentrations and some related antioxidant enzyme activities in patients with cutaneous leishmaniasis. Biol. Trace Elem. Res. 1998; 65: 271-81.

9-A.L. Bertho, M.A. Santiago, A.M. Da-Cruz and S.G. Coutinho. Detection of early apoptosis and cell death in T CD4+ and CD8+ cells from lesions of patients with localized cutaneous leishmaniasis. Brazilian Journal of Medical and Biological Research (2000) 33: 317-25.

10-Fraker PJ, King LE, Laakko T and Vollmer TL. The dynamic link between the integrity of the immune system and zinc status. J. Nutr. 2000; 130 (suppl. 5S): 1399S-1406S.

11-Fernands G, Nair M, Onoe K, Tanaka T, Floyd R and Good RA. Impairment of cell mediated immune function by dietary Zn deficiency in mice. Proc. Natl. Acad. Sci. USA 1979; 76: 457-61.

12-Ruhl H. and Kirchner H. Monocyte-dependent stimulation of human T-cells by Zn. Clin. Exp. Immunol. 32: 484-488.

13-Dardene M, Pleau JM, Nabbara B et al. contribution of zinc and other metal to biological activity of the serum factor. Proc. Natl. Acad. Sci. USA.1982; 79: 5370-3.

14-Coto J.A, Hadden EM, Sauro M, Zom N. and Hadden JW. Interlukin 1 regulates secretion of Zn- thymulin hormone by thymic epithelial and cells and its action on T-lymphocytes proliferation and nuclear protein kinas C. Proc. Natl. Acad. Sci. U.S.A. 1992; 89: 7752-7756.

15-Crea A, Guerin V, Ortega F. and Hartemann P. Zinc and immune system. Ann. Med. Intern. 1990; 141: 447-451.

16-Wellinghausen N. and Rink L. The significant of the Zinc for leukocytes biology. J. Leuk. Bio. 1998; 64: 571-577.

17-Shi HN, Koski KG, Stevnson MM and Scott ME. Zinc deficiency and energy restriction modify immune responses in mice during both primary and challenge of Heligmosomoides polygyrus (Nematode). Parasite Immunol. 1997; 18: 363-372.

18-Cung MT, et al. NMR study of lymphocytic differentiating thymic factor. An investigation of the Zn (2)-non peptide complex (thymolin). J. Biol. Chem.1988; 263:5574-80.

19-Raqib R, Roy KS, Rahman JM, Azim T, Ameer SS, Chisiti J and Andersson J. Effect of Zinc supplementation on immune and inflammatory response in pediatric patients with shigellosis. Res. 2005; 1-3.