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

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ABSTRACT:
Cutaneous leishmanisis still is a common skin infection worldwide and because it is 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-Hawija 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 group 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 confirmation 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±0.01 for 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 الثانية في الليشمانيا الجلدية وعلاقتها مع الخلاصين
حسين ساهر أسود العبيدي - عاطف رفعت - عبد أحمد سالمان - آزاد كمال

المستخلص:
الليشمانيا الجلدية لا تزال من الأمراض الشائعة حول العالم ولأن نوع العصار السرييري للمرض يعتمد على الجهاز المناعي والذي يتأثر بدوره بالمادة والخلايا المناعية. تم دراسة 107 مريضاً بالليشمانيا الجلدية خلال الفترة بين تشرين الأول 2004-نيسان 2005 في قسم الأمراض الجلدية في مستشفى الحوبيط. المرضى تم تقييمهم في مجموعتين تم تقسيمهم لأجل الجهاز المناعي ومعطيات الخلاصين. وشملت الدراسة ستجيني شخصًا معاكساً لمجموعة مريض وتم تقسيم المرضى ومجموعة الضغط إلى مجموعتين: المجموعة الأولي وتم ترخيف شخصين تراوح آعمارهم بين 15-1 سنة ومجموعة الثانية تضم أربعين شخصاً (عشرين أثنا عشرين ذكور). بعد التاريخ والفحص البدني الموافق المرضى. السريري تم تأخير الشخصين من خلال الأجل. تم فحص مستوى الخلاصين المصلية في المرضى فوق عمر 15 سنة كان منخفضاً بالنسبة للفتيات بين 7.61±0.03 ميكرونول/لتر للذكور، و14.08±0.04 ميكرونول/لتر للذكور، و13.03±0.03 ميكرونول/لتر للذكور، و12.02±0.02 ميكرونول/لتر للذكور. ونسبة المريضين من المجموعة البديلة، وكانت معدل نسبة CD4⁺ منخفضاً بالنسبة للفتيات بين 59.95±0.16 % للذكور، و15.15 % للذكور. ونسبة CD8⁺ بين المريضين من المجموعة البديلة 58.9 % من الفتيات، و0.14±0.004 للذكور. وكانت معدل نسبة CD4⁺/CD8⁺ منخفضاً بالنسبة للفتيات بين 31.15±0.15 للذكور، و22.47±0.05 للذكور، و22.37±0.05 للذكور، و32.37±0.05 للذكور.
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. braziliensis and L. mexicana. The cutaneous leishmaniasis of old world is due to L. tropica, L. major, L. aethiopica, L. infantum, and L. donovani. The two species that present in Iraq are: L. tropica, agent of anthropoontic 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 amastigotes. 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 [3].

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 Hawaja general hospital in AL-Hawaja 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 subdivided 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±0.01 for 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 explain what this study revealed that serum Zn concentration in all CL patients is significantly decreased and goes in agreement with what found by [1], 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 [2]. This finding is in agreement with that found by Weyenbergh et al [3] that low serum Zn and iron levels in serum of CL patients in Turkey [4]. 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 [5]. 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. [6], 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. [7], 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. [8], 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. [9] 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. [10] who found that Zn deficiency has effect on proliferation of CD8\(^+\) T-lymphocytes, and is in agreement with that found by Crea et al. [11], that Zn deficiency is associated with decreased T cell proliferation after mitogen stimulation, and is in agreement with Wellingshausen et al., significance of Zn for leukocytes biology, furthermore in agreement with Shi et al. that Zn deficiency affected the phenotypic distribution of splenic T-lymphocytes cells bearing CD3\(^+\), CD4\(^+\), CD8\(^+\). 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. [12], who found that Zn is bound to thymulin hormone in a 1:1 stoichiometry structure and thymulin activity, in vitro and vivo in both
animals and humans is dependent on plasma Zn concentration and thulin hormone responsible to keep the ratio of CD4+/CD8+ in normal range, and is in agreement with that found by Raqib R, et al. [10] that CD4+/CD8+ ratio decreased due to Zn deficiency in child infected with shigellosis. In contrast no significant difference was found between the mean 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 thulin 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

<table>
<thead>
<tr>
<th>Serum Zn Concentration in Above 15 Years Old</th>
<th>Patients μmol/l</th>
<th>Control μmol/l</th>
<th>(P&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>7.61 ± 0.03</td>
<td>14.08 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6.69 ± 0.31</td>
<td>12.02 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

| 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

<table>
<thead>
<tr>
<th>CD4+ Mean in Above 15 Years Old</th>
<th>Patients %</th>
<th>Control %</th>
<th>(P&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>36.5 ± 0.98%</td>
<td>59.95 ± 0.16%</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>36.29 ± 0.06%</td>
<td>58.9 ± 0.14%</td>
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</tbody>
</table>

| CD4+ Mean in Under 15 Years Old | 16.5±0.03% | 44.2±0.13% | (P< 0.05) |

<table>
<thead>
<tr>
<th>CD8+ Mean in Above 15 Years Old</th>
<th>Male 22.37±0.05%</th>
<th>30.95±0.12%</th>
<th>(P&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female 22.47±0.04%</td>
<td>31.15±0.15%</td>
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</table>

| CD8+ Mean in Under 15 Years Old | 16.5±0.04% | 29.5±0.13% | (P< 0.05) |

<table>
<thead>
<tr>
<th>CD4+/CD8+ Mean in Above 15 Years Old</th>
<th>Male 1.63±0.004</th>
<th>1.94±0.01</th>
<th>(P&lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female 1.50±0.004</td>
<td>1.89±0.01</td>
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</tr>
</tbody>
</table>

| CD4+/CD8+ Mean in Under 15 Years Old | 1.44±0.003 | 1.49±0.01 | (P> 0.05) |

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