Assessment of Leishmanin Skin Test and its Relationship With The Clinical Form and Duration of Cutaneous Leishmaniasis

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Abstract
Cellular immunity plays a major role in natural defense against cutaneous leishmaniasis. The leishmanin skin test (LST) is one method of evaluating the infected individual's immune response to leishmania. Our aim in this study was to evaluate the relationship between positivity of the LST with duration of disease, clinical form, number of lesions, and age and gender of the patient. This study was performed on 198 patients who were affected by cutaneous leishmaniasis before any treatment was administered. Following confirmation of the diagnosis of cutaneous leishmaniasis, relevant data were recorded, including age, gender, occupation, address, duration of disease, clinical form, location of the lesions, and the number of the lesions. After performing the leishmanin skin test, patients were treated for leishmaniasis according to the type and severity of the disease. For patients whose LST was initially negative, the test was repeated every 15 days. If the LST was still negative after 4 months, the test was repeated every 3 months; if the LST remained negative 12 months after the first test, the result was considered negative. In 179 patients (90.4 %) the test was positive at the time of the first test. In 7 patients (3.8 %) it became positive during treatment, and in 12 patients (6 percent %) the test remained negative until the end of study. There was no significant relationship between the skin lesion number and the positivity of the leishmanin skin test ($p = 0.98$). There was no significant relationship between age group and diameter of the induration. All of the patients who had a negative leishmanin test at the 12 months follow up visit had one lesion only. This study showed that there is no relationship between age, gender, or duration of disease with positivity of the LST or degree of positivity, but there is a significant relationship with the clinical form of cutaneous leishmaniasis at the final test (12 patients). This study showed that LST positivity did not correlate with the type of treatment.

Introduction
Cutaneous Leishmaniasis of the Old World is widespread in the Middle East and other areas (1). The infected sand fly transmits promastigotes to humans. The parasites are engulfed by tissue macrophage where they multiply as a mastigotes (2). There are at least five species causing Cutaneous Leishmaniasis in the Old World. L.major and L. tropica cause the majority of cases (3). Following an incubation period that is usually up to 12 weeks, one or several lesions appear in the form of one or more red papules that gradually become nodules. Lesions then become crusted, and after 4-12 months, heal with residual scars (3,4).

Although affection by cutaneous leishmaniasis typically induces immunity against the causative parasite, infection with Leishmania major can also induce cross-immunity against Leishmania tropica (5). The leishmanin skin test (LST) is indicative of the delayed-type hypersensitivity to leishmania, which plays a major role in disease resolution and wound healing. This test characteristically becomes positive 5-7 weeks after initiation of infection (6). The test is performed by intradermal injection of 0.1 ml of leishmanin solution. After 24-48 hours, the injection site is inspected and induration is measured. The test is usually considered positive when induration is greater than 5mm (7). A study of the LST showed that an antigen comprising L. major promastigotes gave a sensitivity of 85 percent and specificity of 100 percent (8). Another study has shown that LST-positive leishmaniasis remained positive for 6 months to 3 years (9). In studies assessing the effect of multiple leishmanin skin tests on immune response,
volunteers did not convert to LST positive. Interferon-γ and IL-10 levels remained unchanged throughout the study. Repetition of the LST test does not modulate the in vivo or in vitro immune responses to leishmania antigen (10).

The aim of this study is to evaluate skin test positivity and its relationship to the clinical form of the disease, number of lesions, patient's age, and type of treatment.

Material and methods

This study was performed in the dermatology clinic of Baghdad Teaching Hospital from the period of January 2000 till the end of January 2003. The patients were selected randomly and their disease duration was no more than 3-months (based on the clinical examination). Patients with a history of chronic disease, malnutrition, malignancy, hematologic disorders, atopic dermatitis, or hypersensitivity were excluded from the study.

To perform the test, 0.1 ml of leishmanin antigen was injected intradermally into the anterior surface of the right forearm, and 0.1 ml of the control solution was injected into the anterior surface of left forearm using a 1 ml insulin syringe. After 48 hours, the sites of injection were evaluated for erythema and induration.

If induration in the test area was 5 mm or more and there was no reaction in the control area, the test was regarded as positive; if not, the test was considered negative. If there was a reaction in the control area, the test was regarded as unusual. After performing the leishmanin test, patients were treated for leishmaniasis according to the type and severity of the disease. For patients whose test was negative, the test was repeated every 15 days. If the test was still negative after 4 months, the test was repeated every 3 months, and if it remained negative 12 months after the first test, the result of the leishmanin test was considered negative.

All of the collected data were analyzed by using statistical tests including t-test and Chi square.

Results

Of the 210 patients selected, 102 patients were males and 108 patients were females. The study was completed by 198 of the 210 patients (95 male and 103 female). The age range of patients was 5 months to 70 years, with a mean age of 18.4 years (Figure 1).

At the first testing, the leishmanin test was positive in 179 patients (90.5 %) and negative in 19 patients (9.6 %). There was no significant relationship between age or gender with positivity of the test (p = 0.54) (Figure 2). Of the ten patients with disease duration less than 15 days, eight (80 %) had a positive test and two (20 %) had a negative test.

Of the 90 patients with disease duration 16-30 days, 84 patients (93.3 %) had a positive test and six patients (6.7 %) had negative test. All of the six (100 %) patients whose disease duration was 31-45 days had a positive leishmanin test. Of the 60 patients with disease duration 45-60 days, 52 patients (86.7 %) had a positive test and eight patients (13.3 %) had a negative test. Of the 32 patients with disease duration longer than 60 days, 29 patients (90.6 %) had a positive test and three patients (9.4 %) had a negative test. Therefore, there was no significant relationship between duration of infection and leishmanin test positivity.

There was no significant relationship between the clinical form of the leishmaniasis and the positivity of leishmanin skin test (p = 0.27, see Table 1). There was also no significant relationship between the induration diameter of the leishmanin skin test and the type of the lesion (p < 0.05).

Out of the 179 patients who had a positive leishmanin skin test, the diameter of induration was 5 mm in 122 patients (68.1 %), 6 mm in 31 patients (17.9 %), 7 mm in twelve patients (6.7 %), 8 mm in eleven patients (6.1 %), 9 mm in one patient (0.6 %) and 20 mm in one patient (0.6 %) (Table 2).

Out of the 19 patients who had negative first leishmanin skin test, twelve patients had one lesion, three patients had 2 lesions, and four patients had more than 4 lesions.
In the first leishmanin skin test (198 patients), there was no significant relationship between the skin lesion number and the positivity of the leishmanin skin test ($p = 0.98$) or the duration of the infection and the diameter of the induration ($p = 0.35$).

Of the 19 patients who had an initial negative leishmanin skin test, seven patients became leishmanin test positive (two patients became leishmanin positive after 15 days, one patient after 30 days, one patient after 60 days, one patient after 90 days, and two patients after 120 days). There were twelve patients (7 males and 5 females) who remained leishmanin-test negative after 1 year. Of these, five patients were younger than 6 years old (10% of this age group), three patients were 6-14 years old (5.3% of this age group), and four patients were older than 14 (4.4% of this age group).

Of the twelve LST negative patients, seven had papular leishmaniasis, two patients had nodular leishmaniasis, two patients had plaque-type leishmaniasis, and one patient had nodulo-ulcerative leishmaniasis.

All twelve of the patients who had negative leishmanin tests at the 12-month follow-up visit had only 1 lesion. This finding was statistically significant ($p = 0.013$). Treatments performed for these twelve patients after the initial negative leishmanin test included intramuscular pentavalent antimonial (Pentostam®) for two patients, intralesional pentavalent antimonial for three patients, cryotherapy for four patients, and no treatment for three patients.

Table (1): Relationship between clinical form of the leishmaniasis and the first leishmanin test.

<table>
<thead>
<tr>
<th>Type of the lesion</th>
<th>Total number of lesions:</th>
<th>Positive Leishmanin test No (%)</th>
<th>Negative Leishmanin test No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papule</td>
<td>43</td>
<td>37 (86%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Nodule</td>
<td>30</td>
<td>26 (86.6%)</td>
<td>4 (13.4%)</td>
</tr>
<tr>
<td>Plaque</td>
<td>25</td>
<td>22 (88%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Ulcerative</td>
<td>20</td>
<td>18 (90%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Nodulo Ulcerative</td>
<td>80</td>
<td>76 (95%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Total Lesions</td>
<td>198</td>
<td>179 (90.4%)</td>
<td>19 (9.6%)</td>
</tr>
</tbody>
</table>

Table (2): Relationship between the diameter of induration and age group of the patients.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Diameter of induration</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5mm</td>
<td>6mm</td>
</tr>
<tr>
<td>&lt; 6 yrs</td>
<td>34</td>
<td>5</td>
</tr>
<tr>
<td>6-14 yrs</td>
<td>38</td>
<td>9</td>
</tr>
<tr>
<td>&gt;14 yrs</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>Total Number</td>
<td>122</td>
<td>31</td>
</tr>
</tbody>
</table>

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Discussion

Human leishmaniasis is initiated by the bite of an infected female sandfly and the concurrent intradermal inoculation of the protozoan parasite *Leishmania* spp. Leishmanial parasites are obligatory intracellular pathogens and replicate within macrophages, as well as within dendritic cells and fibroblasts (11, 12, 13).

In lesions of localized cutaneous leishmaniasis (LCL), macrophages and a large numbers of CD4-positive T cells are clustered in the upper part of the dermis, whereas in diffuse cutaneous leishmaniasis (DCL) the inflammatory infiltrate is evenly distributed in the whole dermal compartment. An immunological consequence of this change in the inflammatory infiltrate is the presence of a delayed-type hypersensitivity response against leishmania antigen in LCL but not in DCL patients (14,15). This indicates normal and deficient Th1-type responses, respectively. The leishmanin skin test is representative of the delayed-type hypersensitivity reaction that has a pivotal role in disease resolution and lesional healing. Consequently, this test is usually positive in localized cutaneous leishmaniasis and negative in diffuse cutaneous leishmaniasis. When positive, LST is usually positive 5-7 weeks after infection (6). The LST is usually used as an indicator of the prevalence of leishmania infection in human populations. Our objective in this study was to evaluate the sensitivity and specificity of this test.
Our results showed that, among all patients being tested at the time of presentation and without any therapeutic intervention, 90.4 percent had a positive leishmanin skin test. Of those, 3.86 percent became test positive during treatment and 6 percent (twelve patients) remained test negative until the end of study (1 year). These twelve patients were followed for an additional 2 years but no more leishmaniasis lesions were detected in them despite residing in the endemic area. There was no significant difference in the mean of age of this group compared to the other patients. However, there was a significant relationship regarding the negativity of the test and type of lesions (p = 0.013). All of these twelve patients had just one lesion. There was no relationship regarding the type of treatment and negativity of the leishmanin test.

Because most of the patients with early lesions had positive leishmanin skin tests and the duration of the lesions, sex, and age had no significant impact on the positivity of the test, the use of this simple test can be very helpful for confirming the diagnosis of suspected early lesions. It appears that early treatment may prevent the LST from converting to positive. A negative LST may be attributed to anergic state of the patient, decreased cell-mediated immunity, early treatment, or presence of an unusual serotype of leishmanial parasite.

Except in one patient, all patients with ulcerative lesions had a positive leishmanin skin test. The leishmanin skin test appears to have a very high diagnostic value in patients with ulcerative lesions and therefore can be used in the diagnosis of ulcerative skin lesions suspected to be leishmaniasis.

References