Risk factors for anthroponotic cutaneous leishmaniasis in unresponsive and responsive patients in a major focus, southeast of Iran

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مقدمة و اهداف: لیشمیوزیس جدید یک مشکل جدی در سطح جهانی به دلیل افزایش نرخ انتقال تولید و سایه‌برداری اجتماعی این بیماری در توده‌های درمان ناپاسخ درمانی‌های استرخندی و استرخندی درمانی با گلوکانتمی در بیماران - end stage.

مواد و روش‌ها: این مطالعه از اپریل 2015 تا اکتبر سال 2017 در چندین بیمارستان ایران انجام شد. از ابزارهای اسپارسی و ابزارهای دیگر مورد استفاده در مطالعه استفاده شد. در این مطالعه نتایج نشان داد که درمان به‌صورت گلوکانتمی در 5-12 ماه، درمان درمان ناپاسخ باشد.

بحث و نتیجه‌گیری: بروز سرطان، نوراکسیددی و بالتیکسیون، مایوزیکن ناشی از تولید نورون‌های فعال در بیماری‌های ایمنی مزمن، شامل کانکرسیون و مباینی می‌باشد. این مطالعه نشان داد که درمان ناپاسخ با گلوکانتمی در 5-12 ماه، درمان درمان ناپاسخ باشد.

Leishmania tropica

درمان ناپاسخ با گلوکانتمی در 5-12 ماه، درمان درمان ناپاسخ باشد.

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Leishmania tropica

درمان ناپاسخ با گلوکانتمی در 5-12 ماه، درمان درمان ناپاسخ باشد.
کلمات کلیدی: لیشماتیا تروپیکا، عامل خطر، لیشمانیوزیس جلیلی نوع شهری، بیماران عدم پاسخ به درمان، تفاوت‌های نوکلوتیدی، پاسخ ایمنی، لاوامیزول، گلوکانتم
ABSTRACT

**Background and Objective:** Cutaneous leishmaniasis (CL) is a serious health challenge at the global level due to *Leishmania tropica*. This study was conducted to evaluate the risk factors associated (environmental, nucleotide variations and the host’s immune response factors) with anthroponotic CL (ACL) in unresponsive and responsive patients in a major focus in southeastern Iran. Also, the other objective of the present study was to determine the effect of immunomodulator-Levamisole in combination with Glucantime on end-stage patients with ACL.

**Methods:** This study was conducted from April 2015 to October 2017 in the southeast of Iran. In risk factor evaluation, patients were compared for environmental, clinical, and demographic characteristic factors and were analyzed using multivariate logistic regression and backward elimination stepwise models. $P<0.05$ was defined to be statistically significant. Nucleotide variations survey was carried out in southeastern Iran. Randomly selected skin-scraping lesions of patients (20 non-healed and 20 healed) were examined and the organisms were grown in a culture medium. Promastigotes were collected by centrifugation and kept for further molecular examinations. The extracted DNA was amplified and sequenced. In the host’s immune response study, blood samples were taken from patients and peripheral blood mononuclear cells (PBMCs) were isolated. Two wells were considered for each isolate of unresponsive and responsive patients; one was exposed to *L. tropica* (*Lt*-stimulated) and the other remained non-exposed (non-stimulated). Whole RNA was extracted from each sample. Quantitative real-time PCR was carried out to confirm the differences in expression levels of IL-12 P40, IFN-$\gamma$, IL-1$\beta$, IL-4 and IL-10 among all isolates. Data were analyzed and $p < 0.05$ was considered to be statistically significant. Finally in combination therapy, twenty end-stage patients with ACL were selected for participation in this single-group trial study. Simultaneously, a combination of Levamisole along with Glucantime was used. Several in vitro complementary experiments were performed to evaluate the mode of action of Levamisole and Glucantime alone or in combination.

**Results:** Among the 25 variables, 4 major risk factors including poor interior housing conditions, history of chronic diseases, duration of lesion in the patients referred $\geq 13$ months and 5-12 months than lesions with $\leq 4$ months of age and age groups $\geq 51$ years than those $\leq 7$ years, were significantly associated with unresponsive forms. In molecular study, our results showed that all isolates belonged to the *Leishmania tropica* complex, with their genetic composition in the ITS1 region being different among non-healed and healed patients. 7SL RNA and Hsp70 regions were genetically identical between both groups. Also, in our study, *Lt*-stimulated and non-stimulated unresponsive groups demonstrated significantly lower expression levels of IL-1$\beta$, IL-12 P40 and IFN-$\gamma$ genes and higher expression levels of IL-4 and IL-10 genes, compared to *Lt*-stimulated and non-stimulated responsive groups. Finally in combination therapy, 75% of the patients showed complete clinical cure, 10% partially improved and the remaining (15%) had underlaying chronic diseases demonstrated no response to the treatment regimen.
Conclusion: Improving interior house construction protecting high risk individuals and those with debilitating diseases from being bitten by sand flies, together with the early detection and effective treatment of older age groups with history of chronic diseases are highly important measures for preventing unresponsive forms in patients with ACL in southeastern Iran. Also this study showed that variability in nucleotide patterns observed between both groups in the ITS1 region may serve to encourage future research on the function of these polymorphisms and may improve our understanding of the role of parasite genome properties on patients’ response to Leishmania treatment. Results on the host’s immune response showed that probably, different immune responses caused by various factors play a major role in the pathogenesis and development of unresponsiveness in patients with ACL. The profile and timing of cytokine production correlate with the clinical outcome of Leishmania infection. Finally the findings clearly indicate that the combination of Levamisole and Glucantime should be considered in end-stage patients with ACL who have not responded to basic treatment.

Keywords: Leishmania tropica, risk factor, anthroponotic cutaneous leishmaniasis, unresponsive patients, Nucleotide variations, immune response, Levamisole, Glucantime.
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