Mediterranean Journal of Hematology and Infectious Diseases

Scientific Letter

VCAM-1 and ICAM-1 Serum Levels as Markers of Relapse in Visceral Leishmaniasis

Keywords: Leishmaniasis, VCAM-1, ICAM-1.

Citation: Makis A., Tsabouri S., Karagouni P., Rogalidou M., Sionti I., Chaliasos N. VCAM-1 and ICAM-1 serum levels as markers of relapse in visceral leishmaniasis. Mediterr J Hematol Infect Dis 2017, 9(1): e2017011, DOI: http://dx.doi.org/10.4084/MJHID.2017.011

Published: January 1, 2017 **Received: October 3, 2016** **Accepted: November 18, 2016**

Visceral leishmaniasis (VL), caused by the protozoan parasite *Leishmania donovani* infection, is characterized by a varied spectrum of clinical and laboratory manifestations and by the potentiality of relapses (1,5-10%) despite current therapy.[1] Following infection, many cell adhesion interactions have been identified among monocytes/macrophages, vascular endothelial cells and the parasite;[2,3] and various changes in the expression of adhesion molecules VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intracellular adhesion molecule-1) and L-selectin have been found in experimental VL.[4,5] We hypothesized that the monitoring of this cell to cell interaction system through the course of VL could be useful in estimating the disease progress.

We enrolled in the study 16 children (10 boys, 2-15 years) hospitalized for VL. Most of the children had presented with fever, hepatosplenomegaly, anemia and thrombocytopenia. The clinical diagnosis was confirmed by positive serology, PCR technology or parasite presence in the bone marrow macrophages (**Table 1**). The standard treatment regimen was liposomal amphotericin B (3 mg/kg) on days 1 to 5, 14, and 21. The serum levels of VCAM-1, ICAM-1, and L-selectin were determined in the patients at days 0, 15 and 30, as well as in 20 gender and age-matched healthy children. Commercial ELISA kits were used (Quantikine, R&D Systems, Inc., Minneapolis, USA). Mann-Whitney U test, Wilcoxon matched pairs test and x^2 test were appropriately used for the statistical analysis.

All children recovered completely, while three children relapsed 3, 5 and 6 months after treatment. At day 0, VCAM-1, ICAM-1, and L-selectin were similar to controls ($p>0.05$). At day 15, VCAM-1 and ICAM-1 were significantly increased ($P=0.0012$, $P=0.0032$) where L-selectin remained stable ($P=0.75$). At day 30, VCAM-1 and ICAM-1 decreased at levels comparable to pretreatment values in the 13 children who subsequently had a good outcome without relapses ($P=0.88$), but not in the three patients who relapsed ($P=0.0007$). No differences were noted in L-selectin levels ($P=0.19$) (**Table 2**). The adhesion molecules levels were further analyzed

Table 1. Clinical and laboratory characteristics in 16 children with visceral leishmaniasis at diagnosis.

Clinical symptoms	N (%)
Fever	16 (100)
Sweats	3 (18.7)
Arthralgia/ Arthritis	1 (6)
Myalgia	1 (6)
Malaise	4 (25)
Headache	2 (12.5)
Cough	3 (18.5)
Gastrointestinal symptoms *	5 (31)
Physical findings	
Splenomegaly	13 (81)
Hepatomegaly	10 (62.5)
Lymphanedopathy	3 (18.7)
Skin lesions ǂ	7 (43.7)
Laboratory findings	
Anemia	11 (68.7)
Thrombocytopenia	9 (56)
Leukopenia	8 (50)
Pancytopenia	5 (31)
Elevated inflammatory markers Ŧ	16 (100)
Elevated transaminases	3 (18.7)
Low serum albumin	5 (31)
Positive serology	14 (87.5)
Positive PCR	5 (31)
Parasites in the bone marrow	7 (43.7)

*vomiting, diarrhea, anorexia. ǂ pallor, hemorrhagic rash, jaundice. Ŧ C-reactive protein, erythrocyte sedimentation rate.

for both non-relapsers and relapsers. Non-relapsers showed a significant decline in VCAM-1 and ICAM-1 levels at day 30 (P=0.0006 and P=0.0008) compared to day 15. By contrast, in relapsers day 30 serum VCAM-1 and ICAM-1 had not significantly decreased as compared to day 15 (P=0.45, P=0,72). No differences were demonstrated on day 0, 15 and 30 L-selectin values (**Table 3**). No differences were noted regarding gender, age, symptoms and the laboratory tests on admission, such as hemoglobin, white blood cell counts, platelets, C-reactive protein, erythrocyte sedimentation rate, total protein levels or albumin levels.

These findings may be explained by the cell adhesion interactions during the immune response and the effect of the anti-parasite treatment. The suppressed VCAM-1 and ICAM-1 levels at diagnosis could reflect the adverse effect of *Leishmania* against the adhesive interactions to stop the leukocyte attraction to the site of parasitic infection. The interaction of VCAM-1 with its ligands is crucial for the efficient control of *Leishmania donovani* infection, especially in the liver. Interestingly, the blockade of VCAM-1 leads suppresses anti-leishmania immune responses and leads to higher hepatic parasite accumulation.[6] A similar mechanism of down-regulation of ICAM-1 has been found in human synovial cells in vitro infected with Borrelia burgdorferi.[7] The elevation of VCAM-1 and ICAM-1 in day 15 could be the beneficial effect of liposomal amphotericin B which destroys the parasites and allows the cell to cell interactions. After the end of treatment, the number of tissue parasites dramatically diminishes, and this is probably the reason why the VCAM-1 and ICAM-1 levels return to pre-treatment levels in the children who had a good long-term outcome without relapses. The persistence of high VCAM-1 and ICAM-1 values in the children who relapsed despite they received the same treatment possibly reflects the ongoing immune response to the remaining parasites as well as the action of liposomal amphotericin B. Maybe, these children had a tissue parasite burden larger at front, and they could have taken advantage from the repetition of second treatment schedule. L-selectin did not show any alterations during the disease. One possible explanation is that L-selectin acts on lymphocyte-endothelial cell interactions and activates Th2 (T helper 2) immune response, which is not implicated in the host defense against *Leishmania*.[8]

In conclusion, we found that serum levels of VCAM-1 and ICAM-1 at day 30 post-treatment demonstrated statistically significant correlation with the possibility to relapse in this small group of patients, while L-selectin showed no association. Despite the low number of the patients of this study, our findings indicate that the measurement of VCAM-1 and ICAM-1 during the course of VL may guide and predict disease evolution and outcome in children. Although the mechanisms underlying the association between serum VCAM-1 and ICAM-1 levels and the adverse outcome has not yet been elucidated, further investigation with a larger number of patients would clarify their role as factors of disease severity and confirm their importance as prognostic markers.

Table 2. Serum levels of VCAM-1, ICAM-1 and L-selectin (mean ± standard error of the mean) in children with visceral leishmaniasis and controls.

	Day 0	Day 15	Day 30	Controls
VCAM-1	608 ± 173	982 ± 181	626 ± 84	592 ± 157
ICAM-1	186 ± 92	559 ± 102	192 ± 72	178 ± 88
L-selectin	1022 ± 220	1126 ± 175	1015 ± 186	974 ± 217

Table 3. Serum levels of VCAM-1, ICAM-1 and L-selectin (mean ± standard error of the mean) during treatment in relapsers and non-relapsers.

Treatment outcome	Adhesion molecule	Day 0	Day 15	Day 30
Relapsers (3)	VCAM-1	612 ± 125	992 ± 170	925 ± 131
	ICAM-1	192 ± 77	590 ± 95	538 ± 83
	L-selectin	1035 ± 181	1190 ± 102	1124 ± 125
Non-relapsers (13)	VCAM-1	606 ± 102	965 ± 94	667 ± 115
	ICAM-1	182 ± 79	572 ± 88	217 ± 94
	L-selectin	985 ± 197	1052 ± 173	1144 ± 145

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Competing interests: The authors have declared that no competing interests exist.

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