Türkiye Parazitoloji Dergisi, 34 (3): 156-160, 2010
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Serum Levels of Macrophage Migration Inhibitory Factor and Leptin in Patients with Acute Trichinellosis

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SUMMARY: The aim of this study was to examine the serum levels of MIF and leptin in patients infected with *Trichinella britovi*. Thirtysix patients with acute trichinellosis and 20 healthy control subjects were included in the study. Serum high sensitive C-reactive protein (hs-CRP), MIF and leptin levels were studied. Assessments were performed in the patients group during acute infection and after the treatment periods and we also compared these values with healthy controls. In the patients, hs-CRP, leukocyte and eosinophil counts, and the levels of muscle enzymes in the acute infectious period were found to be significantly higher than those in the post treatment period ($p<0.05$). Both leptin and MIF were higher in acute trichinellosis patients when compared to controls. However, this was only significant for leptin. This study indicates that leptin and MIF levels are increased in the sera of patients with acute trichinellosis.

Key Words: *Trichinella*; Macrophage Migration Inhibitory Factors; Leptin

Akut Trichinellosisli Olgularda Serum Makrofaj Migrasyon İnhibitör Faktör (MİF) ve Leptin Değerlerinin Araştırılması

ÖZET: Bu çalışmada *Trichinella britovi* ile enfekte olan olgularda MIF ve leptin düzeyleri araştırılmıştır. Otuzaltı akut trichinellosis (TT) olgu ve 20 sağlıklı kişi (K) çalışmaya dahil edildi. Hastalarda akut (TA) ve tedavi sonrası (TS) dönemlerde serum örneklerinde hs-CRP, MIF ve leptin düzeyleri ölçüldü, sağlıklı kişilerdeki serum düzeyleriyle de karşılaştırıldı. Hastalarda hs-CRP, lökosit, eozinofil ölçümleri akut enfeksiyon döneminde tedavi sonrası döneme göre yüksek bulundu ($p<0.05$). Leptin ve MIF değerleri akut trichinellosisli hastalarda kontrol grubuna göre daha yüksek saptandı. Kontrol ve olgu grupları arasındaki fark yalnızca leptin için istatistiksel olarak anlamlı bulundu. *Trichinella* varlığına bağlı olarak MİF ve leptin değerlerindeki bu artış hücresel immün yanıtın uyarılmasına bağlı olabileceği düşünüldü.

Anahtar Sözcükler: Makrofaj migrasyon inhibitör faktör, Leptin, *Trichinella*

INTRODUCTION

Trichinellosis is a zoonotic infection caused by parasites of the genus *Trichinella* acquired from eating the muscles of wild or domestic animals (21). Most infections are subclinical and the development of symptoms depends on the number of larvae ingested, the host immune system and the species of parasite (14). Laboratory tests that are often found to be abnormal in trichinellosis include elevated creatine phosphokinase (CPK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels, representting muscle breakdown; elevated total white blood cell count (WBC), eosinophilia and high levels of serum immunoglobulin (Ig) E (29).

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine which has an important role in the regulation of inflammatory and immune responses (22). Lipopolysaccharide and pro-inflammatory effector molecules of immune cells, such as tumor necrosis factor and interferon-gamma are strong inducers of MIF production by macrophages (5). Besides macrophages, T cells, monocytes, blood dendritic cells, neutrophils, B cells, eosinophils, mast cells, basophils and also many normal tissues,

Makale türü/Article type: **Araştırma / Original Research**
Geliş tarihi/Submission date: 18 Haziran/18 June 2010
Düzeltme tarihi/Revision date: -
Kabul tarihi/Accepted date: 26 Ağustos/26 August 2010
Yazışma /Correspoding Author: L. Didem Kozacı
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including the pituitary express MIF (4, 10). Although increased levels of MIF have been reported in several inflammatory and infectious diseases (26) and it has been suggested to play a role in the pathogenesis of some parasitic infections in animals (26, 28), there is no report investigating the relation of MIF with parasitic infections in humans.

Leptin, an adipokine which is an important signal in the regulation of food intake and energy balance (15) has been also shown to be increased during several inflammatory or infectious processes including parasitic infections in animals. However, human studies especially on septic patients have showed controversial results (13) and there is no human study searching the association of leptin with parasitic infections.

In this study, we investigated MIF and leptin levels in sera of patients with trichinellosis during an outbreak of *trichinella britovi*.

PATIENTS AND METHODS

Between December 2003 and January 2004, a large outbreak of trichinellosis, involving more than 600 subjects occurred in Izmir, Turkey (1). It was due to consumption of raw meat ball made with pork infected with *Trichinella britovi*. One hundred and ninety four subjects were admitted to our out-patient clinic during this outbreak and "confirmed acute trichinellosis" was diagnosed in 107 of them, based on the criteria established by Dopouy-Camet et al (11). The present study consisted of 36 of these 107 patients with acute trichinellosis.

Serum hs-CRP, MIF and leptin levels before and after the treatment were investigated in all the patients and compared to those in 20 healthy control subjects. The controls were healthy voluntary blood donors.

Total leukocyte and eosinophil numbers and the levels of muscle enzymes (CPK, AST and LDH) and albumin in sera of the patient group before and after the treatment were also measured.

All the patients were treated with mebendazole (25 mg/kg/day) for 14 days. Prednisolone 20 mg/day was used concomitantly in 24 of them (65%). Control blood samples of the patients were obtained within 5 days after the treatment.

MIF and leptin levels in patients' sera were measured by a specific sandwich cytokine ELISA from R&D (Abingdon, UK) specific for MIF and leptin. Serum high sensitive C-reactive protein (hs-CRP) levels were measured by a sensitive turbidimetric method (Roche Diagnostics). Other laboratory tests including white blood cell (WBC) and eosinophil counts, levels of CPK, LDH and AST were analyzed by routine techniques.

Statistical analysis

The Wilcoxon signed rank test was used for comparison of treatment effects on variables. In comparison of the continuous variables, the Mann-Whitney's U test was used. The Spearman correlation coefficients were calculated for estimation of the level of association between variables. All the statistics were performed using the SPSS version 13.0 statistical package. A p value of < 0.05 was considered as statistically significant.

RESULTS

The patient group consisted of 18 male and 18 female subjects (mean age: 34.7 ± 11.1 years) and there were 11 male and 9 female healthy controls (mean age: 31.4 ± 3.7 years).

In the patients, hs-CRP, leukocyte and eosinophil counts, and the levels of muscle enzymes (CPK, AST and LDH) in the pre-treatment period were found to be significantly higher than those in the post-treatment period ($p<0.05$; median values: 15.6 vs. 0.7 mg/L; 9.95 vs. 8.45 μL; 1.8 vs. 0.86 μL; 259 vs. 82 IU/L; 28 vs.16 IU/L and 506 vs. 376 IU/L respectively). Before the treatment, serum albumin as a negative acute phase reactant was significantly lower than that after the treatment ($p<0.05$; median values: 3.95 vs. 4.35 g/dL) (Table 1).

In comparison of pretreatment (during acute infectious period) and after treatment values, serum leptin showed a significant increase following the treatment ($p<0.05$; median values: 9,9 vs. 13,4 ng/mL respectively). On the other hand, comparison of the pretreatment and after treatment values of MIF revealed no significance although MIF levels increased after the treatment ($p>0.05$; median values: 1,9 vs. 4,1ng/mL respectively) (Table 1).

Baseline (pretreatment, acute infectious period) serum leptin values and hs-CRP levels in the patient group were significantly higher than those in the healthy controls ($p<0.05$; median values: 9,9 vs. 1,1ng/mL for leptin and median values: 15.6 vs. 1.87 mg/L for hs-CRP respectively). Although not reaching significance, MIF levels were higher than that of healthy controls ($p>0.05$; median values: 1,9 vs. 0,9 ng/mL respectively) (Table 2).

Correlation analysis revealed that MIF and leptin levels during acute infection were not correlated with hs-CRP, muscle enzymes (CPK, AST and LDH), serum albumin, eosinophil and leucocyte levels ($p>0.05$). Leptin and MIF were also not correlated with each other ($p>0.05$).

DISCUSSION

In the present study, which is the first investigation exploring serum MIF and leptin levels during a trichinella infection, we found that MIF and leptin levels were increased in sera of patients with acute *trichinella britovii* infection. However this increase was statistically significant only for leptin.

Table 1. Comparison of the study variables during acute period (before treatment) and after the treatment

	Before treatment	After treatment	P Value
Leukocyte count (NR: 4-10.3 µL)	9.95 (4.9-24.4)	8.45 (5.3-17.5)	0.013
Eosinophil count (NR:0.9-6 µL)	1.8 (0.4-7.6)	0.86 (0.4-2.2)	<0.001
CPK (NR:26-167 IU/L)	259 (31-493)	82 (29-356)	0.003
AST (NR:1-32 IU/L)	28 (16-69)	16 (11-37)	0.000
LDH (NR:240-480 IU/L)	506 (260-667)	376 (260-544)	0.001
Albumin (NR:3.5-5.2 g/dL)	3.95 (4-5)	4.35 (3.9-4.9)	0.017
hs-CRP (mg/L)	15.6 (0-58.7)	0.71 (0-17.1)	0.000
MIF (ng/mL)	1,9 (0,3-8,9)	4,1 (8,7-10,4)	0.18
Leptin (ng/mL)	9,9 (3,9-12,6)	13,4 (5,9-13,9)	0.035

Continuous data are presented as median with minimum-maximal values. **NR**= normal range; **AST**= aspartate aminotransferase; **CPK**= creatinine kinase; **hs-CRP**= high sensitive C-reactive protein; **LDH**= lactate dehydrogenase; **MIF**= macrophage migration inhibitory factor

Table 2. Comparison of hs-CRP, MIF and leptin levels in the trichinellosis patients and healthy controls

	Patients during acute infection (n=36)	Controls (n=20)	P
hs-CRP (mg/L)	15.6 (0-58.7)	1.87 (0.21-5)	<0.001
MIF (ng/mL)	1,9 (0,3-8,9)	0,9 (0,7-32,0)	0.6
Leptin (ng/mL)	9,9 (3,9-12,6)	1,1 (0,1-37,1)	0.006

Continuous data are presented as median with minimum-maximal values. **hs-CRP**= high sensitive C-reactive protein; **MIF**= macrophage migration inhibitory factor

Increased MIF levels has been reported in several infectious and inflammatory diseases (26). MIF has been suggested to have an important role in the pathogenesis of several parasitic infections such as malaria, cysticercosis and leishmaniasis in addition to pathogenesis of bacterial and viral infections (17). It acts as a classic pro-inflammatory cytokine promoting innate and adaptive immune responses through the activation of macrophages and T cells. Although both subsets of T cells express MIF, secretion is predominantly increased in activated Th2 clones (2). It is known that protective immune reaction to trichinellosis include T helper subset 2 (Th2) associated cytokine expression and humoral responses (3, 20). Kozaci et al. previously reported similar results as increased MIF levels in patients with acute cutaneous leishmaniasis (18). In this study, although not reaching significance, we found that MIF levels during acute infectious period were higher than the normal controls. Missing statistical significance may be due to small number of patients (lack of power) in this study. Another explanation for this finding may be that MIF may play a role in the local immune response to the parasite. With malaria infection during pregnancy, MIF levels in infected placental plasma have been shown significantly higher than those in the paired peripheral plasma (8). It is possible that examined plasma cytokine levels may not represent the local tissue environment.

We found no correlation between the serum MIF and eosinophil levels in patients during acute infection. The relationship of MIF to recruitment of eosinophils which are an important defense against parasitic diseases is highly provocative (6). Mammalian MIF has not been reported to have eosinophil recruitment activity, although interestingly it is produced by human eosinophils (23, 27). However, homologs of MIF secreted by the nematode parasite have been shown to be involved in activating macrophages and to be sufficient for the recruitment of eosinophils (12).

Increased leptin levels during infection and inflammation strongly suggest that leptin is part of the immune response and host defense mechanisms (19). Its level is acutely increased by many acute phase factors and during bacterial infections (27). Leptin plays an important role in inflammatory process involving T cells and modulates T-helper cells activity in the cellular immune response (7, 9). It was suggested to be a positive acute phase reactant (28). However, some experimental and clinical studies showed decreased levels of leptin during acute infection (9, 16).

There are a few animal studies investigating leptin levels in parasitic infections. In one of them, infection with *Nippostrongylus brasiliensis* in rats resulted in an increased leptin production (25). Leptin levels were also found to be increased in mice infected with *Plasmodium berghei* (24). In the present study, when compared to controls, leptin

production was significantly increased in patients with trichinellosis during acute infection similar to that found in these animal studies.

In conclusion, this study indicates that leptin and MIF levels are increased in the sera of patients with acute trichinellosis. Further comparative and larger studies are needed to determine their exact role in the pathogenesis of parasitic infections.

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