

Sodium stibogluconate (pentostam) remain the drugs of choice in the treatment of all forms of *Leishmania* infection, several studies have been demonstrated that there is an important immunological component in response to antimonial therapy<sup>(9-11)</sup>.

The objective of the present study was to determine the serum cytokines levels of IFN- $\gamma$ , TNF- $\alpha$ , TGF- $\beta$ , IL-1 $\beta$ , IL-6, IL-8 before, during therapy and after healing in Iraqi patients with CL lesions.

## Methods

**Patients:** This study was conducted at Baghdad Teaching Hospital in Baghdad, during the period from January 2008 to March 2009. Eighty people, 60 patients with CL lesion patients and 20 apparently healthy individuals (control), were included. Their age ranged from 5-40 years.

The clinical diagnosis was confirmed by laboratory demonstration of the parasite in the lesions by direct smears. Lesions were cleaned with ethanol, and punctured at the margins with a sterile lancet. Exudate materials was smeared, dried in air and fixed by methanol. The smears were stained with Giemsa's stain and examined by light microscope. Microscopic diagnosis was made when amastigotes were identified in the smears. In order to confirm the diagnosis, the material was also cultured on Novy Mac – Neal –Nicolle (NNN) medium for up to three weeks to detect the leishmanial promastigotes. Antimonial treatment with (pentostam) was given only through the lesions by intralesional injection, 1-3 ml of this drug was injected, and 1-2 doses were given weekly for two months. The patients were checked weekly for healing or recurrence.

**Determination of cytokines:** The blood samples were collected after the diagnostic procedure from healthy subjects, CL patients, during therapy and after three months when they healed from their infection. Ten ml of venous blood was

withdrawn from each individual, and allowed it to clot for 30 minutes at 37 °C. The tubes were then centrifuged for 10 minutes at 4 °C and 2500 rpm. The serum was collected and stored at -20 °C until the time of the serological test. Serum IL-1 $\beta$ , IL-6, IL-8 and TNF-  $\alpha$  levels were determined by ELISA using a quantitative sandwich enzyme immunoassay technique (EASIA kits for IL- $\beta$ , IL-6, IL-8 by Bio source, Europe) and ELISA kits (Mabtech AB, Sweden) for IFN- $\gamma$ , TNF- $\alpha$ , TGF- $\beta$ . All tests were carried out by vigorously following manufacturer instructions. Serum cytokine levels were calculated by interpolating the standard absorbance readings of the test samples calculated from samples of known concentrations supplied with the kits and assayed in parallel. The data were processed using the SPSS PC statistical program (Statistical Package for Social Sciences, PC version 10.0). Initially, mean cytokine levels were compared between groups using Student t-test.

## Results

The serum levels of IFN- $\gamma$ , TNF- $\alpha$ , TGF- $\beta$ , IL-1 $\beta$ , IL-6 and IL-8 in patients responsive and refractory to antimonial therapy (pentostam) as well as normal controls were determined by ELISA before and after treatment were shown in table 1. The results of the pretreatment determinations of the cytokines levels (TNF- $\alpha$ , IL-1 $\beta$ , IL-6 IL-8) in CL patients were higher significantly ( $p < 0.05$ ) than those in normal controls (Table 2). Serum cytokine levels in patients during treatment are listed in table 1 also. CL patients who were responsive to therapy presented with elevated levels of these cytokines ( $p < 0.05$ ) and they were significantly higher than in control group (Table 2). Low levels of IFN- $\gamma$  and TGF- $\beta$  were determined in serum of patients group when compared to control group (healthy), but during therapy these concentrations were increased significantly