Trakia Journal of Sciences, Vol. 12, Suppl. 1, pp 219-222, 2014

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

TNF-ALPHA SERUM LEVELS IN PATIENTS WITH ADVERSE CUTANEOUS DRUG REACTIONS

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ABSTRACT

PURPOSE. The aim of the present study was to investigate the serum levels of TNF-alpha in patients with adverse cutaneous drug reactions (ACDRs).

PATIENTS AND METHODS. Thirteen patients with ACDRs of variable clinical presentation predominantly with maculopapular drug exanthems (MDEs) and twenty six controls were studied. In eight cases the skin eruption was accompanied by systemic symptoms. The causal relationship between drugs and adverse reactions was evaluated with the Naranjo algorithm. Commercial ELISA tests for human TNF-alpha were used.

RESULTS. The causality of ACDRs was rated as "probable" in 4 cases and as "possible" in 9 cases. The serum TNF-alpha level in the patient group was significantly elevated (p=0.023) in comparison with controls. There was no relationship between the presence of systemic symptoms in patients with ACDRs and serum levels of TNF-alpha above the median of the controls (p=0.51).

CONCLUSIONS. TNF-alpha is a versatile cytokine that could be involved in the mechanism of immediate and in T-cell mediated reactions to drugs. MDEs, the most common manifestation of ACDRs have been associated with variable T-cell populations and cytokine patterns. The elevation of serum TNF-alpha in our series of patients comprising predominantly of delayed type ACDRs confirmed a Th_1 cytokine pattern.

Key words: adverse cutaneous drug reaction, TNF-alpha, maculopapular drug exanthems.

INTRODUCTION

Adverse cutaneous drug reactions (ACDRs) are a common problem in hospitalized and outpatients. Clinical manifestation ranges from mild skin eruptions to life-threatening multiorgan diseases and diagnosing may be a difficult task. The prevalent part of ACDRs are hypersensitivity drug reactions. With the progress made in understanding the mechanisms of delayed hypersensitivity, it is now accepted that the clinical presentation of a delayed hypersensitivity reaction depends on the T cells and the cytokine pathways involved (1, 2, 3).

TNF-alpha and IL-6 together with IL-1 are multifunctional cytokines participating in the regulation of immune response and inflammation. IL-6 plays an important role in the acute phase of inflammatory reactions – activation of lymphocytes and cells of inflammation, synthesis of acute phase proteins. TNF-alpha is a pro-inflammatory cytokine produced by mainly by macrophages that enhances their phagocytic properties. It stimulates the production of other pro-inflammatory cytokines such as IL-1 and IL-6 (4). Many other cell types including T cells, mast cells, eosinophils and epithelial cells

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release TNF-alpha. TNF-alpha stored in mast cells is released rapidly at the beginning of the allergic reaction (5). TNF-alpha regulates the secretion of eotaxin, which together with IL-5 participates in the accumulation and activation of eosinophils. TNF-alpha induces programmed cell death (apoptosis) of some cells. It participates in the cell mediated immunity against bacteria, viruses and parasites.

TNF-alpha is necessary for normal immune responses. However it`s overexpression has severe pathological consequences. TNF-alpha participates in the massive keratynocyte apoptosis mediated by FasL and cytotoxic T cells in severe cutaneous drug reactions such as toxic epidermal necrolysis (6, 7).

The aim of the present study was to investigate the serum levels of TNF-alpha in patients with ACDRs.

PATIENTS AND METHODS

Patients

Thirteen patients (5 males and 8 females) with an age range from 23 to 74 years with ACDRs were included in the study. The clinical manifestation of these cutaneous adverse drug reactions was variable: maculopapular drug exanthems (MDEs) in 8 cases, acute urticaria in 3 cases, allergic vasculitis in 1 case, s-ma Stevens Johnson/TEN in 1 case. In 8 cases the skin eruption was accompanied by systemic symptoms - general malaise, fever and edema of epiglottis. In three of these cases the eruption was a part of drug-induced hypersensitivity syndrome. The causal relationship between drugs and adverse reactions was evaluated with the Naranjo algorithm (8). Serving as controls were 26 healthy subjects (16 females and 10 males) with age ranging from 25 to 74 years.

Blood sampling

Blood samples for cytokine assay were collected as part of a routine laboratory check-up of patients with allergic reactions at the time of full blown rash. Blood was collected and allowed to clot at room temperature for 30 minutes before spinning. Serum was aliquoted and stored at -20^0C prior to assessment.

Cytokine Assay

Commersial solid-phase sandwich enzyme immunoassay for human TNF-alpha (Invitrogen Corporation, Camarillo, CA) was used. The sensitivity of the kit TNF-alpha UltraSensitive was above 0.09 pg/ml. ELISA tests were calibrated using recombinant human cytokine standards. Serum samples of cases and controls were analyzed in duplicate together in the same analytic batch.

Statistical analysis

Variables were tested for normality using Shapiro–Wilk test. Mann-Whitney test was used to test for differences between patients and controls. Chi square test was used to compare categorical variables. A value of $p<0.05$ was considered statistically significant. Analyses were performed using SPSS for Windows version 9.0.

RESULTS

The causality of ACDRs was rated as "probable" (Naranjo score 5-8) in 4 cases and as "possible" (Naranjo score 1-4) in 9 cases. The drugs causing the adverse reactions belonged to the group of chemotherapeutic agents (amoxicillin, lincomycin, co-trimoxazole) – 5 cases, the group of NSAIDs (metamizole and ketoprofen) – 5 cases and psychotropic drugs (carbamazepine) in 3 cases.

The median TNF-alpha serum level **(Table 1)** in the group of patients with ACDRs was 14.2 pg/ml (interquartile range 12.19-18.74) while the median level in the control group was 12.93 pg/ml (interquartile range 10.75-14.0). The statistical analysis estimated that the elevation of TNF-alpha serum concentration in the patient group was significant ($p=0.023$) in comparison with the level measured in the control group **(Table1, Figure 1).**

There was no relationship between the presence of systemic symptoms in the patients with ACDRs and TNF-alpha serum levels above the median in the patient group in the acute stage of the disease ($p=0.51$).

DISCUSSION

Drugs can induce immediate type or delayed type hypersensitivity reactions. Maculopapular drug exanthems are the most common manifestation of an allergic reaction to medications (2). They comprise a diversity of clinical, immunologic and histological patterns. MDEs have been attributed to two different categories of type IV reactions: IVb chracterized by T-cells and TH_2 pattern (IL-4 and IL-5

production) and IVc mediated by CD_4 and CD_8 lymphocytes and the production of granzyme B and perforin (1). However some studies support a well defined Th_1 pattern for these reactions (9, 10).

Table 1. *TNF-alpha serum concentrations in patients with ACDRs and controls*

TNF-alpha (pg/ml)	Patients (n=13)	Controls (n=26)	p*
mean ± SD	24.98 ± 36.02	11.59 ± 4.55	
median	14.2	12.93	0.023
interquartile range	12.19 – 18.74	10.75 – 14,0	

n – number of participants; * Mann-Whitney U тест

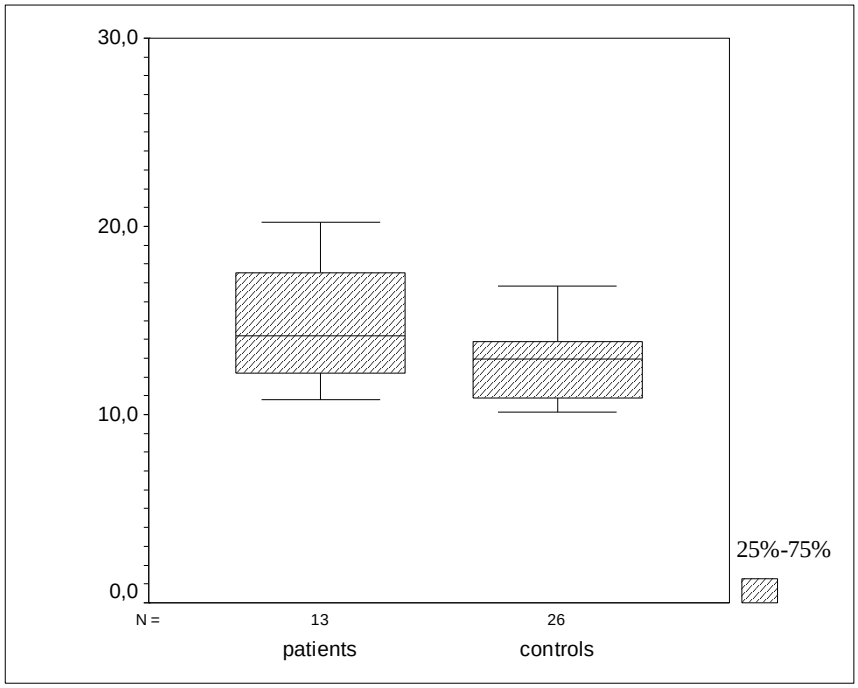

Figure 1. Differences in serum TNF-alpha levels between patients and controls (p=0,023)

TNF-alpha is a versatile cytokine that could be involved in the mechanism of IgE-mediated reactions to drugs (11) but also in the early stages of T-cell mediated reactions to drugs (10). The investigated patients with ACDRs, with the exception of three urticaria cases, presented with delayed type skin reactions to drugs. The elevation of the serum levels of TNF-alpha in our series of patients comprising predominantly of delayed type ACDRs confirmed a Th_1 cytokine pattern. In a similar study (12) the plasma concentration of TNF-alpha and its type I receptor (p55TNF-R) have been found to be significantly higher in the acute stage of drug-induced skin reactions such as maculopapular eruptions, erythema multiforme, hyperergic vasculitis and Stevens-Johnson syndrome. In the investigated group of patients blood samples were taken only in the acute stage of the disease. According to some authors neither peak, nor mean serum levels of cytokines are decisive for the degree of damage they induce, but the time duration for cytokine increase (13). The kinetics of TNF-alpha serum levels in various stages of drug-induced disease might be much more informative of its role in the development of ACDRs. For definite conclusions more patients and repeated blood sampling in the acute stage and during recovery are required. We found no relationship between the presence of systemic symptoms that are indicative of a more severe course of ACDRs, and the TNF-alpha serum levels above the median, a fact which is also consistent with the idea that the severity of drug-induced damage is dependent not only on the increased levels of cytokines including TNF-alpha and other pro-inflammatory cytokines but, perhaps, on the time duration of cytokine increase.

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