Serum Levels of Interleukin-2 in Patients with Alopecia Areata: Relationship with Clinical Type and Duration of the Disease

Emina Kasumagić-Halilovic^a Semra Cavaljuga^b Nermina Ovcina-Kurtovic^a
Lamija Zecevic^c

^aDepartment of Dermatovenereology, Clinical Centre of Sarajevo University, Sarajevo, Bosnia-Herzegovina;

^bInstitute of Epidemiology and Biostatistics, Faculty of Medicine, University of Sarajevo, Sarajevo,

Bosnia-Herzegovina; ^cInstitute of Clinical Immunology, Clinical Centre Sarajevo, Sarajevo, Bosnia-Herzegovina

Keywords

Alopecia areata · Autoimmunity · Immunoassay · Interleukin-2

Abstract

Background: Alopecia areata (AA) is a disease characterized by focally, nonscarring hair loss on the scalp or any hair-bearing surface. The etiology is unknown, although the evidence suggests that AA is an immunologically mediated disease. In the pathogenesis of AA, Th1 immune response is predominant. A special cytokine profile is created by Th1 cells, which disturbs the natural balance of the cytokine networks and leads to inflammatory reaction and follicle damage. **Objective:** The aim of our study was to evaluate serum concentrations of IL-2 in patients with AA and healthy subjects. We also examined a possible association between serum levels of IL-2, disease severity, and duration of AA. **Methods:** Sixty patients with AA and 20 healthy controls were enrolled in the study. Serum concentrations of IL-2 were measured using enzyme-linked immunoassay techniques. **Results:** Comparison of mean values of IL-2 has showed that serum concentrations of this cytokine are significantly higher in serum sam-

ples of AA patients in relation to the control group (22.2 ± 1.19 vs. 21.1 ± 2.68 pg/mL, respectively; $p = 0.0142$). No correlations were found between clinical type, duration of the disease, and serum levels of IL-2. **Conclusion:** Our findings support the evidence that elevation of serum IL-2 is associated with AA. The exact role of serum IL-2 in AA should be additionally investigated in future studies.

© 2018 S. Karger AG, Basel

Introduction

Alopecia areata (AA) is a heterogeneous disease characterized by nonscarring hair loss on the scalp or other parts of the body. It affects 1–2% of the population of both genders and occurs in all age groups [1]. A wide range of clinical presentation can occur – from a single patch of hair loss (alopecia unilocalis, AU), multiple patches (alopecia multilocalis, AM), to complete loss of hair on the scalp (alopecia totalis, AT) or the entire body (alopecia universalis, AU). AA is a systemic disease because, in addition to hair follicles, it can also affect the nails and the eyes.

Although, the etiopathogenesis of the disease is not clear, several studies have shown that within the cascade of the pathogenesis of AA, the cytokines play a crucial role. It is also considered that a disequilibrium in the production of cytokines, with a relative excess of proinflammatory versus anti-inflammatory cytokines, may be involved in the persistence of AA lesions [2–4]. Hair loss may occur because proinflammatory cytokines interfere with the hair cycle, leading to the premature arrest of hair cycling with cessation of hair growth [5]. This concept may explain typical clinical features of AA, such as a progression pattern in centrifugal waves and spontaneous hair regrowth in concentric rings, suggesting the presence of soluble mediators within affected areas of the scalp [6]. T cells cloned from the lesional scalp also produce cytokines that inhibit the proliferation of keratinocytes, which may provide an additional mechanism for interference with the anagen phase of the hair cycle [7].

Interleukin-2 (IL-2) was discovered in 1976, as a growth factor for T lymphocytes [8]. Since that time, it has become an important mediator of immune function through its effects on the growth, development, and activity of T and B lymphocytes, natural killer cells, and lymphokine-activated killer cells [9]. Apart from its most important role in mediating antigen-specific T-lymphocyte proliferation, IL-2 modulates the expression of interferon- γ and major histocompatibility antigens. Changes in serum IL-2 levels have been found in many diseases, such as vitiligo [10], systemic lupus erythematosus, and psoriasis [11]. In some of these diseases, serum IL-2 concentrations correlated with activity and intensity of the disease, and may be used as a prognostic factor. Because of the central role of IL-2 in immune response, IL-2 turned out to be a very important molecule for diagnostic and therapeutic implications.

Although it is well known that multiple cytokines simultaneously play a role in AA, many authors have measured only one particular cytokine. Our study has focused only on IL-2 because there are only a few studies that have measured the serum levels of this cytokine, with controversial results. Therefore, the aim of our study was to evaluate serum concentrations of IL-2 in patients with AA and healthy subjects, and also to assess a possible association between IL-2 and clinical type and duration of the disease.

Materials and Methods

Patients

The study was conducted in the Department of Dermatovenereology at the University Clinical Centre Sarajevo, Sarajevo, Bosnia and Herzegovina. All subjects gave their informed consent in

accordance with the requirements of the Institutional Ethics Committee.

The study included 60 patients (36 female and 24 male, median age 35.6 years, ranging from 5 to 69 years) who contacted the Dermatovenereology Clinic with complaints of hair loss and were diagnosed with AA. Patients who had received any treatment within the previous 3 months were excluded from the study, as well as patients with any diseases based on the immune pathomechanism, which could influence serum concentrations of IL-2. According to the clinical type of AA, patients were divided into 4 groups: patients with (1) AUI, (2) AM, (3) AT, and (4) AU. According to the duration of disease, the patients were divided into 3 groups: duration of (1) 6 months or less, (2) more than 6 months but less than 12 months, and (3) 1 year or longer. The control group consisted of 20 generally healthy people (11 female and 9 male, age range 6–63 years, median age 32.6 years).

IL-2 Measurement

Serum concentrations of IL-2 were measured by an enzyme-linked immunosorbent assay (ELISA) technique, using a Quantikine Human IL-2 Immunoassay (R&D Systems, Minneapolis, MN, USA). Briefly, a monoclonal antibody specific for IL-2 is precoated onto a microplate. Standards and samples are pipetted into the wells and any IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Statistical Analysis

The data are expressed as mean $\pm$ standard deviation. The test distribution was calculated by the Kolmogorov-Smirnov test, and comparisons were performed by the Mann-Whitney U test. The data were considered statistically significant if *p* values were less than 0.05 (5%) level. Statistical analyses were performed using the SPSS software.

Results

The study group was composed of 60 patients diagnosed with AA (36 female and 24 male; mean age 35.6 years, range 5–69 years) and 20 healthy controls (11 female and 9 male; mean age 32.6 years, range 6–63 years). There were no significant differences in age and female/male ratio between the patients and controls (*p* > 0.05). The mean duration of AA was 14.5 ± 25.4 months (range 1–119). In the total of 60 patients with AA, there were 13 (21.6%) patients with AUI, 33 (55%) with AM, 7 (11.7%) with AT, and 7 (11.7%) with AU.

In our study, the mean serum IL-2 level in AA patients was 22.2 ± 1.19 pg/mL (range 19.5–26.0), with 5% of variation. Figure 1 shows the individual values of IL-2 in both the AA and control groups.

Table 1. Serum concentrations of IL-2 in patients with alopecia areata (AA), divided according to clinical types of the disease, in comparison with the control group

Characteristics	Total AA	Alopecia unilocularis	Alopecia multilocularis	Alopecia totalis	Alopecia universalis	Control group
Patients, <i>n</i> (%)	60 (100)	13 (21.6)	33 (55.0)	7 (11.7)	7 (11.7)	20
Age, years						
Mean $\pm$ SD	35.6 $\pm$ 16.80	34.7 $\pm$ 20.30	36.5 $\pm$ 16.5	33.7 $\pm$ 13.46	34.7 $\pm$ 17.30	32.6 $\pm$ 16.12
X _{min}	5	10	5	16	11	6
X _{max}	69	68	69	53	57	63
Duration, months						
Mean $\pm$ SD	14.5 $\pm$ 25.4	11.1 $\pm$ 25.83	11.99 $\pm$ 23.4	15.6 $\pm$ 12.7	31.9 $\pm$ 38.8	–
X _{min}	0.33	0.33	0.33	2.0	3.0	–
X _{max}	120.0	96.0	120.0	36.0	108.0	–
IL-2, pg/mL						
Mean $\pm$ SD	22.2 $\pm$ 1.19	22.4 $\pm$ 1.60	22.0 $\pm$ 1.21	22.5 $\pm$ 0.34	22.2 $\pm$ 0.70	21.1 $\pm$ 2.68
X _{min}	19.5	19.5	20.0	22.0	21.3	10.6
X _{max}	26.0	25.6	26.0	23.1	22.1	23.8
K _v	0.05	0.07	0.05	0.01	0.03	0.13

Fig. 1. Individual values of IL-2 in alopecia areata (AA) patients and controls.

According to clinical type AU, AM, AT, and AU, the mean serum IL-2 ranged from 22.00 to 22.5 pg/mL (22.4, 22.00, 22.5, and 22.00 pg/mL, respectively), with variation of K_v = 7, 5, 1, and 3%. The highest IL-2 concentrations were observed in patients with AT. Patients with longer duration of the disease had a higher concentration of IL-2, but not significantly ($p > 0.05$).

In order to prove the correlation, the Pearson coefficient of correlation was calculated. This resulted in $r = -0.252$. As the investigated variables were not distributed by normal distribution, it was necessary to measure Spearman's rank correlation coefficient ρ (rho) for hypothesis testing, resulting in $\rho = -0.3313$ (95% CI -0.0844 ; 0.5398). Regarding the results of the confidence intervals, with $p = 0.010$, we concluded that the correlation between duration of disease and concentration of IL-2 is not significant. Among controls, IL-2 mean was measured to be 21.1 ± 2.68 pg/mL (range 10.6–23.8), with 13% of variation.

Statistical analysis showed significant differences in IL-2 values between patients with AA and healthy controls ($U = 380$, $z = 2.451$, $p = 0.0142$). Comparison among the 4 groups (according to the clinical type of AA) did not show any statistically significant differences ($p > 0.05$) (Table 1).

Discussion

Although the pathogenesis of AA is still poorly understood, a perifollicular, peribulbar, and perivascular accumulation of T lymphocytes provides the evidence that an immune process is involved, interfering with the hair cycle and leading to irreversible hair loss. Recent progress in the understanding of AA has shown that the regulation of

local and systemic cytokines plays an important role in its pathogenesis [12, 13]. The immune response presented in AA is associated with aberrant lesional expression of interferon- γ , IL-2, and IL-1 β , and overexpression of ICAM-1 and major histocompatibility complex molecules on hair follicle keratinocytes and dermal papilla cells [14]. Several clinical and experimental findings point towards IL-2 as a crucial mediator inducing immunologic reaction against human follicle in AA [15, 16]. By using semiquantitative reverse transcription-polymerase chain reaction with RNA extracted from scalp biopsies from patients with AA, Hoffmann et al. [17] reported that IL-2 mRNA levels were increased in untreated AT.

In addition, increased IL-2 serum levels in patients with AA compared with normal controls have been reported, further suggesting a role for this cytokine. In the study of Teraki et al. [18], the researchers compared the serum levels of cytokines, including IL-1 α , IFN- γ , IL-2, and IL-4, in patients with the localized and extensive form of AA, and found that the serum levels of IL-1 α and IL-4 were significantly elevated in patients with the localized form. In contrast, the serum levels of IFN- γ and IL-2 were significantly elevated in patients with the extensive form. The researchers stated that these findings could be interpreted as an indication that Th1-type cytokines might be critical for progression to the extensive form and that Th2-type cytokines may exert a more subtle influence of a cell-mediated attack on hair follicles. Barahmani et al. [19] analyzed the serum cytokine profiles in 269 patients with AA and found that increased IL-2 levels were associated with AA regardless of the disease severity. Their study suggests that there is a Th1 response early in the disease, as patients with transient AA had higher serum IL-2 levels than patients with longer duration of the disease.

The results presented in our study demonstrated that the mean serum levels of IL-2 were significantly elevated in AA patients, in comparison with healthy subjects.

There was no significant difference in IL-2 levels between the patients with localized and extensive forms. Patients with longer disease duration had a higher concentration of IL-2, but not significantly. Our findings are similar to the study by Tembhre and Sharma [20], who also recorded a significant increase in IL-2 serum in patients with AA, and IL-2 levels were positively correlated with the total disease duration and number of patches on the scalp. The abovementioned researchers concluded that the increased levels of serum IL-2 suggested altered T-helper cell function. To the contrary, in a study carried out in Russia, a significant lowering of IL-2 was noted in patients with severe AA [21]. The authors hypothesized that lowering of IL-2 in patients with severe forms of AA may be an indirect confirmation of the participation of autoimmunity mechanism in the pathogenesis of these forms of AA and implies an increase of disbalance in immunoregulatory cells.

In conclusion, our findings that the serum levels of IL-2 were significantly elevated in patients with AA indicate that IL-2 may play a role in the pathogenesis of AA. In this study we assume that the elevated serum levels of IL-2 may reflect the inflammatory symptoms in AA, and that control of IL-2 production may be important to the management of this disease. This research could contribute to the interpretation of the insufficiently well-known views of the role and significance of IL-2 in the pathogenesis of AA.

Statement of Ethics

Written informed consent was obtained from the patients, and the study was performed according to the Declaration of Helsinki.

Disclosure Statement

The authors declare no conflicts of interest.

References

- 1 Safavi KH, Muller SA, Suman VJ, Moshel AN, Melton LJ: Incidence of alopecia areata in Olmsted County, Minnesota, 1975 through 1989. *Mayo Clin Proc* 1995;70:628–633.
- 2 Kuwano Y, Fujimoto M, Watanabe R, Ishiura N, Nakashima H, Ohno J, Yano S, Yazawa N, Okochi H, Tamaki K: Serum chemokine profiles in patients with alopecia areata. *Br J Dermatol* 2007;157:466–473.
- 3 Gilhar A, Paus R, Kalish RS: Lymphocytes, neuropeptides, and genes involved in alopecia areata. *J Clin Invest* 2007;117:2019–2027.
- 4 Giordano CN, Sinha AA: Cytokine pathways and interactions in alopecia areata. *Eur J Dermatol* 2013;23:308–318.
- 5 Ito T, Tokura Y: The role of cytokines and chemokines in the T-cell-mediated autoimmune process in alopecia areata. *Exp Dermatol* 2014;23:787–791.

- 6 Del Rio E: Targetoid hair regrowth in alopecia areata. The wave theory. *Arch Dermatol* 1998; 134:142–143.
- 7 Thein C, Strange P, Hansen ER, Badsgaard O: Lesional alopecia areata T lymphocytes downregulate epithelial cell proliferation. *Arch Dermatol Res* 1997;289:384–388.
- 8 Morgan DA, Ruscetti FW, Gallo R: Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 1976;193: 1007–1008.
- 9 Liao W, Lin JX, Leonard WJ: Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. *Immunity* 2013; 38:13–25.
- 10 Singh S, Singh U, Pandey SS: Serum concentration of IL-1, IL-2, TNF- α , and IFN γ in vitiligo patients. *Indian J Dermatol* 2012;57:12–14.
- 11 Roussaki-Schulze AV, Kouskousis C, Petinaki E, Klimi E, Zafiriou E, Galanos A, Rallis E: Evaluation of cytokine serum levels in patients with plaque-type psoriasis. *Int J Clin Pharmacol Res* 2005;25:169–173.
- 12 Bodemer C, Peuchmaur M, Fraitag S, Chatenoud L, Brouse N, Prost Y: Role of cytotoxic T cells in chronic alopecia areata. *J Invest Dermatol* 2000;114:112–116.
- 13 Kasumagic-Halilovic E, Ovcina-Kurtovic N: Serum levels of interleukin-1 (IL-1 α , IL-1 β) in patients with alopecia areata. *Our Dermatol Online* 2012;3:161–163.
- 14 Konig A, Happle R, Hoffmann R: IFN- γ induced HLA-DR but not ICAM-1 expression on cultured dermal papilla cells is downregulated by TNF- α . *Arch Dermatol Res* 1997;289: 466–470.
- 15 Brajac I, Gruber F, Petroveckii M, Malnar-Dragojevic D: Interleukin-2 receptor α -chain expression in patients with alopecia areata. *Acta Dermatovenerol Croat* 2004;12:154–156.
- 16 Valsecchi R, Imberti G, Martino D, Cainelli T: Alopecia areata and interleukin-2 receptor. *Dermatology* 1992;184:126–128.
- 17 Hoffmann R, Wenzel E, Huth A, Van Der Steen P, Schaufele M, Hanninger HP, Happle R: Cytokine mRNA in alopecia areata before and after treatment with the contact allergen diphenylcyclopropenone. *J Invest Dermatol* 1994;103:530–533.
- 18 Teraki Y, Imanishi K, Shichara T: Cytokines in alopecia areata: contrasting cytokine profile in localized form and extensive form (alopecia universalis). *Acta Derm Venereol* 1996; 76:421–423.
- 19 Barahmani N, Lopez A, Babu D, Hernandez M, Donely SE, Duvic M: Serum T helper cytokine levels are greater in patients with alopecia areata regardless of severity or atopy. *Clin Exp Dermatol* 2010;35:409–416.
- 20 Tembhre MK, Sharma VK: T helper and regulatory T cell cytokines in the peripheral blood of active alopecia areata. *Br J Dermatol* 2013;424:27–32.
- 21 Koubanova A, Gadjlgoroeva A: On the problem of pathogenetic heterogeneity of alopecia areata. *European Hair Research Society (EHRS) Conference Abstracts, Brussels, 2002*, p 13.