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Serum levels of neopterin in patients with lichen planus

Lichen planus (LP) is a chronic pruritic inflammatory dermatosis that is commonly associated with mucosal involvement and rarely with nail dystrophy and scarring alopecia (3, 8). The prevalence of lichen planus is unknown, but it is estimated to occur in less than one percent of the population (7). Skin lesions of lichen planus may be described using the six 'Ps': pruritic, polygonal, planar (flat-topped), purple papules and plaques (7). Although the cause is not known, there has been much research into the immune and pathological mechanisms that underlie lichen planus. The dermal infiltrate consists predominantly of helper T cells (4) particularly in active LP lesions at both the dermo-epidermal junction and deeper in the dermis, along with increased numbers of S-100-positive Langerhans' cells and FXIIIa dendritic cells in the epidermis and the dermis (1). The production of cytokines is dramatically increased in lesional LP with raised levels of interleukin (IL)-1β, IL-4, Il-6 and tumor necrosis factor (TNF) (15). Gamma interferon (IFN-γ) is produced by activated lymphocytes, which in turn causes keratinocytes to express HLA-DR antigens and progress into lesional cells (3). Keratinocyte damage and basal cell liquefaction may be mediated by cytokines and the recruitment of cytotoxic T cells, which follows early T cell activation (8).

Neopterin (NP) is a 2-amino-4-hydroxy-(1'2'3'-trihydroxypropyl)-pteridine (5) with low molecular mass (253 Da) [12]. It is produced by activated monocytes/macrophages from guanosine triphosphate (GTP) via GTP cyclohydrolase I (13). The activity of this enzyme is greatly enhanced by INF-γ (10), and, to a lesser degree, by INF-α, other cytokines and endotoxins (2). INF-γ, released by activated helper T lymphoctes type 1 (Th1), and natural killer cells (NK), is the most potent inducer of NP production, and the concentration of NP indicates the presence of INF-γ in body fluids (2). Because INF-γ is released by active T cells, NP is a sensitive marker of cell-mediated immunity (2, 11, 13).

Recent studies have revealed abnormal NP concentrations in the body fluids in various clinical states, such as allograft rejection, infections, autoimmune diseases, malignancies, heart and kidney failure, coronary artery disease and myocardial infarction (2). Increased serum NP levels can be of clinical value in the diagnosis and prognosis of conditions associated with cell-mediated immunity (2). There is a limited number of reliable biomarkers which can be used in the diagnosis and prognosis for lichen planus although it is of importance to monitor the course of this disease. If significantly elevated serum neopterin levels were found these might presumably be used as a reliable laboratory marker reflecting clinical activity of lichen planus. The aim of our study was to assess the serum levels of neopterin in patients with lichen planus and to investigate whether serum neopterin levels reflect extensive lesions and progression of this disease.

MATERIAL AND METHODS

We studied 66 patients with lichen planus (age range 18 to 70 years; mean age 52 ± 2.96 years) who were admitted between 2000 and 2002 to the Outpatient Clinic and the Department of Dermatology, Venerology and Pediatric Dermatology, Medical University in Lublin. The control group consisted of 30 healthy sex-and age-matched individuals (age range, 20 to 67; mean age 49.42 ± 2.20). All patients and controls gave written, informed consent before study entry, and the study was approved by the local Ethics Committee. At the time of enrollment, patients and controls were excluded from the study if there was for at least 8 weeks evidence of infectious disease or medication (i.e. methotrexate, cyclosporin A, retinoids or folates) before. These factors could influence the serum level of neopterin. A diagnosis of lichen planus was reached on the basis of the typical clinical appearance and a punch biopsy of the skin and/or mucosal lesions. The patients with lichen planus were classified into one of two diagnostic categories: Group I comprised 33 patients with generalized lichen planus, with involvement of the upper and lower extremities as well as the trunk; Köbner phenomenon was observed in 30 patients; mucosal involvement of the oral cavity was observed in 17 patients and genital lesions were observed in one patient.

Group II comprised 33 patients with circumscribed lichen planus; the lesions involved the upper extremities (33 patients), and/or the lower extremities (25 patients), and/or the trunk (four patients); Köbner phenomenon was observed in 11 patients; mucosal involvement of the oral cavity was observed in 20 patients and genital lesions were observed in one patient.

Peripheral blood was collected early morning (7.00–8.00 a.m.). Samples were centrifuged, and serum samples were frozen at -70°C until the time of assay. The serum neopterin concentrations were measured with a commercially available enzyme-linked immunosorbent assay kit (EIA 1476; DGR Instruments Gmbh, Germany). Sensitivity of detection of the assay was 0.2 ng/mL. The upper limit of the normal range is approximately 2.5 ng/mL serum (i.e. 10 nmol/L) (11, 13). All results were expressed by means ± standard error of the mean. The Mann-Whitney U-test was used to test for statistical significance of differences of laboratory variables between the different groups; p values of < 0.05 were regarded as significant.

RESULTS

The levels of serum neopterin in both patients with lichen planus and control group are summarized in Table 1. The results from the assay demonstrate that serum neopterin concentrations in group I were significantly higher than in normal controls (9.12 ± 4.39 ng/mL vs 2.55 ± 0.34 ng/mL respectively; $p < 0.001$). There were significantly higher serum neopterin levels in group II than in normal controls (3.80 ± 0.68 ng/mL vs 2.55 ± 0.34 ng/mL respectively; $p < 0.001$). Serum neopterin concentrations differed significantly between group I and group II (9.12 ± 4.39 ng/mL vs 3.80 ± 0.68 ng/mL; $p < 0.01$). There were significantly higher serum neopterin levels in whole collections of patients (group I and group II) than in normal controls (6.55 ± 2.30 ng/mL vs 2.55 ± 0.34 ng/mL respectively; $p < 0.001$).

Table 1. Serum neopterin concentrations in patients with lichen planus and healthy controls

Studied group	Number of individuals (n)	Serum neopterin concentrations (ng/mL)	
		Mean value (x)	Standard error of the mean (SEM)
Control group (healthy controls)	30	2.55	0.34
Group I (generalized lichen planus)	33	[1,2] 9.12	4.39
Group II (circumscribed lichen planus)	33	[1,2] 3.80	0.68
Group I and group II (lichen planus)	66	[1] 6.55	2.30

[1] $p < 0.001$ in comparison with control group
[2] $p < 0.01$, group I in comparison with group II

DISCUSSION

The etiology of lichen planus is unknown, although many studies have investigated and support immunologic pathogenesis. Lymphocytes, particularly T-cells, play a major role. Other factors include antigen-presenting cells, adhesion molecules and inflammatory cytokines. Because NP is secreted by monocytes and macrophages, mainly as a response to INF-γ secretion by activated T-lymphocytes, NP is a sensitive marker of cell-mediated immunity (2, 9, 11, 13). For serum neopterin, the concentration of 2.5 ng/mL (i.e. 10 nmol/L) is generally accepted as upper limit of normal (2, 9). In our study, the neopterin concentrations found in serum samples from healthy control subjects (2.55 ± 0.34 ng/mL) were in good agreement with literature data. The serum neopterin levels in the patients with lichen planus classified to group I and group II as well as the whole collection of patients (group I and group II) were significantly higher than those of the control subjects. Our findings confirm a role for cell-mediated immunity in the pathogenesis of lichen planus. Monocytes/macrophages seem to be the main source of neopterin in humans (2). It has been demonstrated that the production of NP is induced mainly by INF-γ and, to a lesser degree, by INF-α, TNF, granulocyte/macrophage growth factor (GM-CSF) and IL-12 (2, 10). On the other hand, NP stimulates TNF gene expression, which enhances TNF synthesis (6). Although NP increase is observed in many clinical states associated with predominance of Th1 lymphocyte activation, a correlation with IL-6 has also been observed. Thus, neopterin determination is a valuable indicator of the activation of the cell-mediated immune system, although the biological reason for neopterin production by interferon-γ-stimulated human monocytes/macrophages is unknown (2). Most recent experiments have indicated that neopterin acts as an enhancer of several reactions involving oxygen and chlorine free radicals; because these highly reactive substances participate in the effector functions of monocytes/ macrophages, such a role would be compatible with a large amount of data accumulated on the use of neopterin as an immune activation marker (2).

According to the present state of knowledge, NP is released to body fluids (serum, cerebrospinal fluid, synovial fluid, pancreatic juice, urine, saliva and ascites fluid) and then excreted unchanged via kidneys (2, 9, 13). No NP receptor is known and it does not undergo extravasation, which makes it a good indicator of the amount of INF-γ produced (2). Blood NP concentrations are age-dependent, being higher in children and elderly people, but they are not related to gender (2). NP measurements seem to be clinically useful in cardiology for differentiating between unstable coronary artery disease and non-Q-wave myocardial infarction, in transplantology for detecting cardiac and renal graft rejection,

and in the assessment of autoimmune disease course and activity (Graves' disease, Sjögren syndrome and rheumatoid arthritis) (2, 9, 13, 14). Our findings suggest that the serum NP level may be a useful marker of enhanced cellular immunity as well as macrophages activation observed in lichen planus. In our study, the serum levels of neopterin in patients with generalized lichen planus (group I) were significantly higher than in patients with circumscribed lesions (group II). Thus, NP concentrations may serve as predictors of disease progression. It is important for the dermatologist to have an understanding of the pathogenic factors involved in LP, recognize the implications of certain variants of LP and undertake sympathetic, but accurate, detailed observation of patients with this disease.

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SUMMARY

Lichen planus (LP) is a chronic dermatosis whose clinical features include mildly erythematous to violaceous flat-topped, polygonal papules. The etiology of lichen planus is unknown, but it has been postulated that immune mechanism is important. Although it is of importance to monitor the course of immune-mediated diseases, there is a limited number of reliable biomarkers which can be used for lichen planus. Neopterin (NP), a 2-amino-4-hydroxy-(1'2'3'-trihydroxypropyl)-pteridine, is secreted

by monocytes and macrophages, mainly as a response to INF-γ secretion by activated T-lymphocytes. Therefore NP may be a sensitive marker of T-cell mediated immunity. The aim of the presented study was to assess the serum levels of neopterin in patients with lichen planus and to investigate whether serum neopterin levels reflect extensive lesions and progression of this disease. We studied 66 patients with lichen planus who were classified into one of two diagnostic groups: Group I comprised 33 patients with generalized lesions; group II comprised 33 patients with circumscribed lesions. The control group consisted of 30 healthy sex- and age-matched individuals. The serum neopterin concentrations were measured with a commercially available enzyme-linked immunosorbent assay kit. The results of our study are summarized in Table 1. In our study, the serum neopterin levels in the patients with lichen planus classified to group I (9.12 ± 4.39 ng/mL) and group II (3.80 ± 0.68 ng/mL) as well as in the whole collection of patients (group I and group II; 6.55 ± 2.30 ng/mL) were significantly higher than those of the control subjects (2.55 ± 0.34 ng/mL). Our findings confirm a role for enhanced cellular immunity as well as macrophages activation observed in lichen planus. It seems to us that evaluation of serum neopterin levels, despite the relatively low specificity, reflects extensive lesions and lichen planus progression.

Stężenie neopteryny w surowicy krwi u chorych na liszaj płaski

Liszaj płaski jest przewlekłą dermatozą, której obraz kliniczny charakteryzuje się występowaniem wielobocznych, błyszczących grudek barwy od różowej do sinoczerwonej. Etiologia choroby nie jest do końca poznana, a najbardziej prawdopodobne wydaje się tło immunologiczne. Jakkolwiek w chorobach o podłożu immunologicznym ważne jest monitorowanie wybranych parametrów układu odpornościowego, to jednak u pacjentów z liszajem płaskim istnieje ograniczona liczba tego typu markerów o ustalonej wartości diagnostycznej. Neopteryna jest 2-amino-4-hydroksy-(1'2'3'-trihydroksypropylo)-pterydyną produkowaną w monocytach/makrofagach w odpowiedzi na stymulację interferonem-(INF)-γ pochodzącym z aktywowanych limfocytów T. Dlatego jest ona uważana za niespecyficzny marker aktywacji odpowiedzi komórkowej zależnej od limfocytów T. Celem przedstawianej pracy było wykazanie, czy ocena stężenia neopteryny w surowicy krwi potwierdza wzmożoną odpowiedź komórkową w liszaju płaskim, a także czy wzrost stężenia neopteryny odzwierciedla stopień zaawansowania zmian. Badaniem objęto grupę 66 pacjentów z rozpoznanym liszajem płaskim, których podzielono na 2 grupy: grupa I obejmowała 33 chorych ze zmianami uogólnionymi; do grupy II zaliczono 33 chorych ze zmianami ograniczonymi. Grupa kontrolna składała się z 30 zdrowych osób. Badany materiał stanowiły surowice, w których oznaczano stężenie neopteryny, posługując się komercyjnym zestawem do oznaczeń metodą ELISA. Rezultaty naszych badań przedstawione są w tab. 1. Poziomy neopteryny w surowicy krwi pacjentów z liszajem płaskim zaklasyfikowanych do grupy I (9,12 ± 4,39 ng/ml), jak również do grupy II (3,80 ± 0,68 ng/ml), a także w całej grupie pacjentów z liszajem płaskim (6,55 ± 2,30 ng/ml) były istotnie wyższe niż w zdrowej grupie kontrolnej (2,55 ± 0,34 ng/ml). Wyniki naszych badań potwierdzają udział aktywacji makrofagów i wzmożonej odpowiedzi komórkowej obserwowanej w liszaju płaskim. Wydaje się, że określanie poziomów neopteryny w surowicy, pomimo względnie niskiej swoistości, odzwierciedla zaawansowanie choroby i rozległość zmian.