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RELATIONSHIP OF LICHEN PLANUS, HEPATITIS VIRUS C AND LOW LEVEL OF TOTAL ANTIOXIDANT CAPACITY

Simona Roxana Georgescu[1], Alina Musetescu[1], Corina Daniela Ene[2], Cosmin Ene[3], Mădălina Mitran, Cristina Mitran, Mircea Tampa, Ilinca Nicolae
[1]Victor Babes Clinical Hospital of Infectious and Tropical Diseases, Dermatology Clinic, Bucharest;
[2]Carol Davila Clinical Hospital of Nephrology, Bucharest;
[3]Sf Ioan Emergency Hospital, Clinic of Urology, Bucharest
Corresponding author: CSP1 Dr Ilinca Nicolae
Victor Babeș Clinical Hospital of Infectious and Tropical Diseases, Dermatology Clinic - Research,
drnicolaei@yahoo.ro

Rezumat

Pe baza celor mai noi achiziţii din literatura de specialitate, se apreciază că lichenul plan este o afecţiune inflamatorie, asociată cu dezechilibre autoimune, infecţii cu virusul hepatitei C, stres oxidativ, deficit de antioxidanţi. Scopul lucrării prezente este reprezentat de determinarea unui panel de antioxidanţi serici, posibil implicaţi în declanşarea/persistența bolii. Evaluarea profilului unor antioxidanţi extracelulari (bilirubina, acidul uric, albumina, fierul, transferina, feritina, cuprul, ceruloplasmina, potenţialul antioxidant global) la pacienţii cu lichen plan, în timpul exacerbării leziunilor, a evidenţiat reducerea semnificativă a sistemelor antioxidante non-enzimatice. Virusul hepatitei C accentuează deficitul de antioxidanţi la pacienţii cu lichen plan.

Pe baza acestor constatări, autorii apreciază că lichenul plan este o boală complexă, cu cauze adesea neidentificate şi cu mecanisme etiopatogenice incomplet elucidate. Se poate admite că în declanşarea şi evoluţia lichenului plan ar putea fi intricate mai multe mecanisme care se potenţează reciproc.

Cuvinte cheie: *lichen plan, virusul hepatitei C, antioxidanţi serici.*

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Abstract

Based on the latest medical research, it is supposed that lichen planus is an inflammatory disorder, associated with autoimmune diseases, hepatitis C infection, oxidative stress or antioxidant deficiency. The purpose of the present work is to determine a panel of serum antioxidants, possibly involved in the development/persistence of the disease. The determination of extracellular antioxidants (bilirubin, uric acid, albumin, iron, transferrin, ferritin, copper, ceruloplasmin, total antioxidant capacity) in patients with lichen planus during exacerbations have revealed a significant reduction in non-enzymatic antioxidant systems. Hepatitis C virus enhances the deficit of antioxidants in patients with lichen planus. Based on these findings, the authors consider that lichen planus is a complex disease of unidentified cause and its pathogenic mechanisms are still incompletely elucidated. It may be speculated that several interconnected mechanisms are involved in the onset and evolution of lichen planus.

Keywords: *lichen planus, hepatitis C, serum antioxidants.*

Introduction

Although lichen planus is an intensely studied topic, until now the mechanisms of occurrence and development of this pathology have not been completely elucidated. Lichen planus is a disease that can involve skin, scalp, nails, and mucosal membranes (oral, nasal, laryngeal, esophageal, conjunctival, anal, and genital). It affects between 0.22 and 5% of the general population[1]. Various factors were incriminated in the pathogenesis of lichen planus. Some studies are focused on a possible autoimmune involvement in the basal layer of keratinocytes, other studies define a possible association of lichen planus with the hepatitis C virus (HCV), or a possible association with the administration of certain medicines[1-8]. It may be speculated that several interconnected mechanisms are involved in the onset and evolution of lichen planus.

In a retrospective study, the authors have reported a series of general conditions associated with lichen planus. Of these we note: liver dysfunctions, kidney dysfunctions, metabolic alterations, urinary tract infections[1]. In literature there are similar descriptions regarding the

relationship between lichen planus and liver manifestations (virus C hepatitis, primary biliary cirrhosis), autoimmune diseases (ulcerative colitis, lupus erythematosus, vitiligo, alopecia areata, dermatomyositis, morphea, lichen sclerosus, myasthenia gravis), diabetes mellitus, neoplasias, hypertension, infections (HCV, HSV), urinary lithiasis, stress[1-8].

Some publications show the reduction of antioxidant systems in urine, saliva, blood, and leukocytes in patients with lichen planus[9-23]. A recent analysis of the authors regarding the status of ascorbic acid in patients with lichen planus shows that ascorbic acid has low values in these compared to healthy individuals. According to the obtained results, the presence of bacterial or viral infections identified in the studied groups causes a significant reduction of ascorbic acid in the urine of these patients. Low values of ascorbic acid and presence of nitrites could be useful in detecting people with risk of developing urinary tract infections. Ascorbic acid acts as a catalyst to transform nitrites in chemical species responsible for increasing anti-infective activity[1,9].

Many non-enzymatic components with antioxidant potential were identified in the body[9-22]. Among these a special attention was given to glutathione, lipoic acid, uric acid, albumin, transferrin, ferritin, lactoferrin, ceruloplasmin, vitamins (A, E, C), minerals (iron, copper, manganese, zinc, selenium).

The limits of knowing of the manner in which this pathology occurs and evolves lead to an intensification of the efforts for a better standardization of the protocols to monitor and manage patients with lichen planus. In this paper the authors wished to analyze the possible relationship between the antioxidant capacity of the serum and the activity of the disease, via:

- determination of the biological status of patients diagnosed with lichen planus before starting treatment;
- assessment of the profile of some serum antioxidants and the global antioxidant potential of the serum (TAS) in patients with lichen planus during exacerbation of the lesions, in the pre-therapy phase;
- analysis of statistical differences between the level of the analytes quantified in patients with lichen planus stratified by the presence of HCV infection;
- assessment of correlations between the level of individual antioxidants and the TAS value for patients with lichen planus.

Material and method

This study was conducted with the approval of the Ethics Committee of the Dermatology Clinic of the Dr Victor Babes Clinical Hospital of Infectious and Tropical Diseases, Bucharest, and the informed consent of patients was obtained.

Study participants. A retrospective study was conducted on a group composed of 77 patients with lichen planus and 50 healthy volunteers. All patients were assessed clinically, paraclinically and by imaging. The two groups were similar regarding: residence environment, occupation, gender, age groups. The biological characteristics of patients and controls were summarizes in Table 1.

Inclusion criteria: optimal nutritional intake, adults, with normocalcemia

Exclusion criteria: use of vitamin supplements, treatment with corticosteroids or

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immunosuppressive agents, malabsorption, alcoholics, smokers, children, elderly, dialyzed, infusionable, Whipple's disease or irritable bowel syndrome, anemia, uricosuria, pregnancy, breastfeeding, allergy, diseases of the skeletal system, coagulation disorders, physical and mental strain, infectious diseases, surgeries.

The laboratory determinations were performed from serum, obtained from venous blood collected in vacutainer without anticoagulant, maintained for 30 minutes at room temperature and centrifuged at 6000 rpm for 10 minutes. The supernatant was used for biochemistry and serology determinations.

Determination of serum bilirubin was performed by spectrophotometric method (600nm) using diazotized sulfanilic acid. Determination of uric acid was performed spectrophotometrically (520nm) using an enzymatic analysis method. Determination of albumin was performed by spectrophotometric method (600nm) using the reaction between bromocresol and serum albumin. Dosing of iron was performed spectrophotometrically (623nm) using chromazurol S. Dosing of transferrin was performed immunoturbidimetrically (340nm), using the reaction between transferrin and a specific antiserum.

Dosing of ferritin was performed immunoturbidimetrically (340nm), using the interaction between ferritin and the specific antiserum. Determination of copper was performed by photometric method (580nm) using 3,5 di-Br-PAESA4-(3,5 dibromo-2-pyridylazo)-N-ethyl-N-(3-sulfopropyl) aniline. Dosing of ceruloplasmin was performed by turbidimetric method (340 nm) based on the reaction between ceruloplasmin as antigen and a specific antiserum as antibody. Determination of TAS was performed by spectrophotometric method (600nm) based on the reaction between ABTS (2,2-azino-bis-3-ethylbenzothiazoline-sulfonate) and peroxidase. Detection of HCV was performed by serologic techniques of quantification of anti-HCV antibodies.

Statistical analysis. Comparison of the experimental results between groups for quantitative variates was performed using t-tests or ANOVA. Correlations between variates were established by linear regression. The presence of the relationship between the two parameters was assessed by the Pearson correlation coefficient (r). We have chosen a significance threshold (p) of 0.05 (5%), the confidence level of 95% showing that the decision was just.

Results

Following the detailed anamnesis, clinical examination, paraclinical tests and imaging assessments, 77 patients diagnosed with

lichen planus were selected. In these, systemic manifestations were assessed. The profile of some serum antioxidants (bilirubin, uric acid, albumin, iron, transferrin, ferritin, copper, ceruloplasmin, and TAS) was analyzed in patients with lichen planus compared to the control group (Table 1). In the group of patients with lichen planus, the bilirubin concentration was 0.22±0.08 mg/dL, and in the control group it was 0.39±0.28 mg/dL. No statistically significant variations were seen between the serum bilirubin concentrations in the two groups (p>0.05). Serum uric acid concentrations did not have statistically significant variations between patients and control (3.6±0.8 mg/dL, 4.1±0.6 mg/dL, p>0.05). Regarding the variation of serum albumin concentration in relation to the study groups, a relationship at the limit of statistical significance was found between the serum albumin level in patients with lichen planus versus control (4.02±0.61 g/dL, 4.20±0.65 g/dL, p=0.051).

In the group of patients with lichen planus, the serum iron level was 78.5±21.3µg/dL, and in the control group it was 81.2±17.1 µg/dL. No statistically significant variations were recorded between the serum iron concentrations in the two groups (p>0.05). Serum transferrin concentrations did not have statistically significant variations between patients and control (236.2+-64.3mg/dL, 244.1+_29.8mg/dL, p>0.05). Regarding the variation of serum ferritin concentration in relation to the study groups, a relationship at the limit of statistical significance was found between the serum ferritin level in patients with lichen planus versus control (69.4±32.3 ng/mL, 46.1±28.6 ng/mL, p=0.052).

In the group of patients with lichen planus, the copper concentration was 82.9±17.4 µg/dL, and in the control group it was 81.7±14.8 µg/dL. No statistically significant variations were recorded between the serum copper concentrations in the two groups (p>0.05). Serum ceruloplasmin concentrations did not have statistically significant variations between patients and control (32.1±4.1 mg/dL, 32.7±1.6 mg/dL, p>0.05). Regarding the variation of TAS concentrations in relation to the study groups, a statistically significant difference was found between the serum TAS levels in patients with lichen planus versus control (1.19±0.47 mmol/L, 1.28±0.29 mmol/L, p<0.05).

Subsequently the 77 patients were subdivided in two subgroups: lichen planus with negative serology for HCV (71 cases), and lichen planus with positive serology for HCV (6 cases).

In these subgroups the possible statistical differences between the quantified analytes were assessed. No notable differences were found between the values of bilirubin, uric acid, albumin, iron, transferrin, ferritin, copper, ceruloplasmin, determined experimentally in patients with lichen planus with/without HCV (Table 2).

A special analysis was applied to differences recorded between the variations of TAS in the two subgroups with lichen planus. The serum TAS concentrations had statistically significant variations between patients with lichen planus and negative serology for HCV and patients with lichen planus and positive serology for HCV (1.26±0.52 mmol/L, 1.11±0.33 mmol/L, p<0.05).

Subsequently the relationship between the level of individual antioxidants and the global value of serum antioxidant potential (TAS) was analyzed (Table 3). In patients with lichen planus and negative serology for HCV the following statistical relationships were found: a weak positive association at the

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Biologic parameters	Lichen planus	Control	p
Women/Men	1.42	1.28	0.621
Age (years)	49.5±22.6	51.2±16.5	0.296
Systolic BP (mmHg)	129±8	122±12	0.505
Diastolic BP (mmHg)	69±9	66±11	0.366
Bilirubin (mg/dL)	0.22±0.08	0.39±0.28	0.109
Uric acid (mg/dL)	3.6±0.8	4.1±0.6	0.622
Albumin (g/dL)	4.02±0.61	4.20±0.65	0.051
Iron (µg/dL)	78.5±21.3	81.2±17.1	0.106
Transferrin (mg/dL)	236.2±64.3	244.1±29.8	0.171
Ferritin (ng/mL)	69.4±32.3	46.1±28.6	0.052
Copper (µg/dL)	82.9±17.4	81.7±14.8	0.651
Ceruloplasmin (mg/dL)	32.1±4.1	32.7±1.6	0.822
TAS (mmol/L)	1.19±0.47	1.28±0.29	0.032

Table 1. *Biologic status of study participants*

Variate	Lichen planus (HCV negative)	Lichen planus (HCV positive)	p
Bilirubin (mg/dL)	0.26±0.09	0.34±0.21	0.198
Uric acid (mg/dL)	3.8±0.9	3.6±0.8	0.396
Albumin (g/dL)	4.12±0.55	3.87±0.75	0.054
Iron (µg/dL)	81.5±11.3	77.2±14.6	0.417
Transferrin (mg/dL)	246.2±74.3	224.1±31.8	0.202
Ferritin (ng/mL)	59.4±34.2	76.1±24.7	0.066
Copper (µg/dL)	80.9±11.4	88.7±17.8	0.239
Ceruloplasmin (mg/dL)	31.1±2.1	34.0±2.3	0.061
TAS (mmol/L)	1.26±0.52	1.11±0.33	0.042

Table 2. *Profile of some serum antioxidants in patients with lichen planus stratified by the presence of HCV infection.*

limit of statistical significance between the variations of albumin and TAS (r=0.112, p=0.050), a weak negative association statistically non-significant between serum iron and TAS (r=- 0.149, p>0.05), between ferritin and TAS (r=- 0.103, p>0.05), and between ceruloplasmin and TAS (r=-0.148, p>0.05).

In patients with lichen planus and positive serology for HCV the following relationships were found: a statistically significant positive association between the variations of albumin and TAS (r=0.301, p<0.050), a moderate statistically non-significant negative association between serum cooper and TAS (r=- 0.269, p>0.05), and between ceruloplasmin and TAS (r=-0.298, p>0.05).

Discussions

Cells contain a complex network of antioxidant systems, capable to prevent the oxidative degradation of cell structures[22]. During the last years it was noted that in the skin there is a wide variety of antioxidant factors, and therefore the investigation of the biological effects of free radicals generated a particular interest. In the skin the following were described: ferritin (in cytoplasm), transferrin, lactoferrin, ceruloplasmin, albumin (in extracellular fluid), vitamin E, ubiquinone, carotene (in cell membrane), vitamin C (in cytoplasm), glutathione (in cytoplasm and mytochondria), uric acid and bilirubin (in blood), heme oxygenase-1 (in dermis), heme oxygenase-2, catalase, superoxide dismutase (in epidermis)[22]. It is known that the presence of free radicals causes a decrease of the immune response (immunosuppression), that results in the decrease of the defense capacity of the body against various stimuli.

In this study we performed an analysis of the profile of serum antioxidants in patients with lichen planus in the active phase of the disease, trying to identify the impact of HCV infection on the exacerbation of this disease.

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Pair variates	Lichen planus (HCV negative)	Lichen planus (HCV positive)	Control
Bilirubin / TAS	R=0.031 P=0.237	R=0.063 P=0.488	R=0.012 P=1.00
Uric acid / TAS	R=0.093 P=0.766	R=0.087 P=0.967	R=0.003 P=0.999
Albumin / TAS	R=0.112 P=0.050	R=0.301 P=0.039	R=0.009 P=1.000
Iron / TAS	R= - 0.149 P=0.142	R= - 0.305 P=0.211	R=0.007 P=1.000
Transferrin / TAS	R=0.034 P=0.376	R=0.024 P=0.902	R=0.054 P=0.876
Ferritin / TAS	R= - 0.103 P=0.167	R= 0.178 P=0.187	R=0.011 P=0.992
Copper / TAS	R=0.051 P=0.587	R= - 0.269 P=0.254	R=0.003 P=1.000
Ceruloplasmin / TAS	R= - 0.148 P=0.087	R= - 0.298 P=0.064	R=0.007 P=1.000

Table 3. *Statistical correlations between TAS and the level of some serum antioxidants.*

The decrease of TAS, signaled in a significant proportion in both patient with lichen planus and control, and also in patients with lichen planus and positive serology for HCV versus patients with lichen planus and negative serology for HCV, could be a condition associated to this disease.

From the analysis of the results presented in this paper we can identify two possible pathways of decrease of the TAS level in this pathology.

Firstly, the alteration of the optimal levels of copper and iron in patients with lichen planus and positive serology for HCV leads to disturbance of some processes at both cellular and systemic level. Obtaining of negative correlations between TAS and iron, and TAS-ferritin, respectively, and also between TAS and copper, and TAS-ceruloplasmin, respectively in patients with lichen planus supports the role played by these active redox metals in the destabilization of cellular redox potential. TAS offers information regarding the serum capacity to inactivate reactive radical species, by capturing free radicals and sequestration of transition metal ions, thus preventing the Fenton reaction.

Secondly, a statistically significant positive relationship between TAS and albumin in patients with lichen planus, a correlation expressed particularly in the disease associated with HCV, highlights the role played by the sulfhydryl groups in the decrease of the antioxidant potential in the extracellular space in these patients. The TAS value could be a predictive factor of a relapse episode of lichen planus.

For now the exact mechanisms by which the antioxidant systems form the basis of the exacerbation of this pathology are not known, but there is a constant interest of the specialists for a better standardization of protocols to follow-up and diagnose patients with lichen planus. The authors think that additional studies are required, on a number of patient as high as possible, to define the contribution of antioxidants in the understanding of the pathogenesis of lichen planus. Determination of TAS could be an important criterion to assess the association between lichen planus, HCV infection, and decreased antioxidant capacity of the human serum. The literature offers few information regarding the status of serum antioxidants in lichen planus[1-9]. Decrease of uric acid, inactivation of antioxidant enzymes, decrease of non-enzymatic antioxidants, and of the total antioxidant capacity support the occurrence of an oxidants/antioxidants imbalance in lichen planus.

The pathophysiology changes in the basal layer, in epithelial cells, and in the dermis-epidermis interface reconfirm the relationship between oxidative stress and pathogenesis of lichen planus. Therefore the quantification of non-enzymatic serum antioxidants could be useful in establishing a therapeutic strategy and monitoring of patients with lichen planus.

Conflict of interests: not stated.

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