SERUM ANTIOXIDANT STATUS IN LICHEN PLANUS PATIENTS

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Summary

Based on the most recent developments in literature, lichen planus is considered an inflammatory disease, associated with autoimmune imbalances, hepatitis C virus infection, oxidative stress, antioxidant deficiency. The objective of this paper is to establish a panel of serum antioxidants which play a putative role in disease onset/persistence. The assessment of several extracellular antioxidants (bilirubin, uric acid, albumin, iron, trans-ferrin, ferritin, copper, ceruloplasmin, total antioxidant capacity) in lichen planus patients during lesion flare showed a significant impairment of non-enzymatic anti-oxidant systems. Hepatitis C virus enhances antioxidant deficiency in lichen planus patients. Based on these findings, the authors deem lichen planus to be a complex disease, whose causes often remain unidentified and pathogenesis has not been cleared up. The potential involvement of several mutually enhancing mechanisms in the onset and progression of lichen planus is acceptable.

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

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Introduction

Although lichen planus is heavily studied, the mechanisms involved in the onset and progression of the disease continue to be unclear. Lichen planus is a disease which may affect the skin, the scalp, the nail and mucous membranes (oral, nasal, laryngeal, esophageal, conjunctival, anal, genital) with a prevalence of 0.22 to 5% in the general population (1). A series of trigger factors have been indicated in lichen planus development. While some studies focused on a possible autoimmune involvement in basal keratinocytes, other studies describe a potential correlation between lichen planus and hepatitis C virus (HCV) or a possible association with some medicinal products (1–8). The potential involvement of several mutually enhancing mechanisms in the onset and development of lichen planus is acceptable.

The authors of a retrospective study reported several general medical conditions associated with lichen planus of which we mention: liver impairment, kidney impairment, metabolic changes, urinary tract infections (1). There are similar reports in literature of the association between lichen planus and liver conditions (hepatitis C, primary biliary cirrhosis), autoimmune disease (ulcerative colitis, lupus erythematosus, vitiligo, alopecia areata, dermatomyositis, morphea, lichen sclerosus, myasthenia gravis), diabetes, cancer, hypertension, infections (HCV, HSV), urolithiasis, stress (1–8).

Some articles confirm the impairment of antioxidant systems in urine, saliva, blood and white blood cells in lichen planus patients (9–23). A recent analysis performed by the authors, regarding the status of ascorbic acid in these patients indicates low levels as compared to

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healthy individuals. The findings show that bacterial or viral infections identified in the studied groups cause a significant decrease of ascorbic acid in the patients' urine. Low levels of ascorbic acid and the presence of nitrites may be useful in detecting individuals with a risk to develop urinary tract infections. Ascorbic acid is a catalyst in the transformation of nitrites in chemical species responsible with increased activity against infections (1, 9). Multiple non-enzymatic components with antioxidant capacity have been identified in the body (9–22). Among these, glutathione, lipoic acid, uric acid, albumin, transferrin, ferritin, lactoferrin, ceruloplasmin, vitamins (A, E, C), minerals (iron, copper, manganese, zinc, selenium) have received special attention.

The limited understanding of how this disease occurs and develops steps up efforts towards the optimal standardization of protocols for the follow-up and management of lichen planus patients. In this paper, the authors aim to examine the possible relation between the antioxidant capacity of serum and disease activity by:

- determination of the biological status of patients diagnosed with lichen planus before treatment is started;
- assessment of serum antioxidant status and serum total antioxidant capacity (TAC) in lichen planus patients during lesion flare, before treatment;
- examination of statistical differences of the level of analytes determined in lichen planus patients stratified based on HCV infection status;
- examination of associations between individual antioxidant level and TAC level in lichen planus patients.

Material and methodology

This study was conducted following the agreement of the Ethics Committee of the Dermatology Clinic from the "Prof. Dr. Victor Babeș" Clinical Hospital for Infectious and Tropical Disease – Bucharest; the patients provided their informed consent.

Study subjects. A retrospective study on a group of 77 lichen planus patients with multiple lesions and 50 healthy subjects was conducted. All patients underwent clinical exams and lab tests as well as imaging studies. Characteristics such as place of resi-dence, occupation, gender, age were similar between the groups. The biological charac-teristics of patients and case control subjects are summarized in Table 1.

Inclusion criteria: optimal nutritive intake, adults, blood calcium levels within the normal range.

Exclusion criteria: vitamins supplements use, steroid or immunosuppressive treatment use, malabsorption, alcohol abuse, smoking, children, elderly, dialysis use, perfusion use, Wipple disease or irritable bowel syndrome, anaemia, uric acid in urine, pregnant or lactating women, allergies, bone system diseases, coagulation disorders, physical and mental exhaustion, infectious diseases, surgical procedures.

Venous blood serum samples collected in anticoagulant-free Vacutainer tubes, which were left for 30 minutes at room temperature and centrifuged at 6000 rpm for 10 minutes were used for lab tests. The supernatant was used for biochemistry and serology testing.

Serum bilirubin determination was performed through spectrophotometry (600 nm), with diazotized sulfanilic acid.

Uric acid determination was performed through spectrophotometry (520 nm), using the enzymatic assay.

Albumin determination was performed through spectrophotometry (600 nm), using the reaction between bromocresol and serum albumin.

Spectrophotometry (623 nm) was used for iodine determination, using chrome azurol S.

Immunoturbidimetry (340 nm) was used to determine transferrin, using the reaction between transferrin and a specific antiserum.

Ferritin determination was performed through immunoturbidimetry (340 nm), using the reaction between ferritin and a specific antiserum.

Copper was measured using photometry (580 nm), with 3,5 di-Br-PAESA4-(3,5 dibromo-2-pyridylazo)-N-ethyl-N-(3-sulfopropyl)aniline.

Turbidimetry (340 nm) was used to measure ceruloplasmin levels, based on the reaction between ceruloplasmin as antigen and a specific antiserum as antibody.

TAC was measured by spectrophotometry (600 nm), based on the reaction between ABTS (2,2'azino-di-3-ethylbenzothiazoline sulphonic acid) and peroxidase.

HCV determination was performed through serological techniques for the measurement of anti-HCV antibodies.

Statistical analysis. T tests were used to compare intergroup experimental results for quantitative variables. The correlations between variables were established through linear regression. The Pearson (r) correlation coefficient was used for the description of the correlation between two parameters. We opted for a 0.05 significance threshold (p) (5%), while the 95% confidence level proved that the decision was valid.

Results

Following a thorough medical history evaluation, clinical exam, lab tests and imaging studies, 77 patients diagnosed with lichen planus were selected. They were assessed to detect systemic signs and symptoms. Several serum antioxidant status (bilirubin, uric acid, albumin, blood iron, transferrin, ferritin, copper, ceruloplasmin and TAC) was assessed in lichen planus patients versus the control group (Table 1). In the lichen planus patients group, bilirubin level reached 0.22±0.08 mg/dL, and 0.39±0.28 mg/dL in the control group. No statistically significant differences of serum bilirubin levels were noticed between the two groups (p>0.05). Statistically significant differences did not exist related to serum uric acid levels between the two groups (3.6±0.8 mg/dL, 4.1±0.6 mg/dL, p>0.05). As far as the serum albumin level variations are concerned, a borderline statistically significant correlation was noticed between serum albumin in lichen planus patients versus control subjects (4.02±0.61g/dL, 4.20±0.65 g/dL, p=0.051).

In lichen planus patients, blood iron level was 78.5±21.3 ug/dL, while in the control group it reached 81.2±17.1 ug/dL. No statistically significant variations of serum iron levels were noticed between the two groups (p>0.05). Blood transferrin levels did not reach statistically significant variations between patients and control subjects (236.2±64.3 mg/dL, 244.1±29.8 mg/dL, p>0.05). Serum ferritin levels variations showed a borderline statistically significant correlation between serum ferritin in lichen planus patients versus control (69.4±32.3 ng/mL, 46.1±28.6 ng/mL, p=0.052).

Table 1. Biological characteristics in study participants

Biological characteristic	Lichen planus	Control	p
Female/Male	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 (ug/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 (ug/dL)	82.9±17.4	81.7±14.8	0.651
Ceruloplasmin (mg/dL)	32.1±4.1	32.7±1.6	0.822
TAC (mmol/L)	1.19±0.47	1.28±0.29	0.032

In lichen planus patients, copper level was 82.9±17.4 ug/dL, while in the control group it reached 81.7±14.8 ug/dL. No statistically significant variations were noticed as far as intergroup copper levels are concerned (p>0.05). Serum ceruloplasmin levels did not display statistically significant variations between patients and control subjects (32.1±4.1 mg/dL, 32.7±1.6 mg/dL, p>0.05). The TAC level variations between the study groups displayed a statistically significant difference of serum TAC in lichen planus patients versus control subjects (1.19±0.47 mmol/L, 1.28±0.29 mmol/L, p<0.05).

Afterwards, the 77 patients were divided in two groups: lichen planus with negative serologic test results for HCV (71 cases) and lichen planus with positive serologic test results for HCV (6 cases). Potential statistical differences of measured analytes were assessed. No relevant differences were found in terms of bilirubin, uric acid, albumin, iron, transferrin, ferritin, copper, ceruloplasmin experimentally measured in lichen

Table 2. Serum antioxidant status in lichen planus patients stratified based on HCV infection status

Variables	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 (ug/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 (ug/dL)	80.9±11.4	88.7±17.8	0.239
Ceruloplasmin (mg/dL)	31.1±2.1	34.0±2.3	0.061
TAC (mmol/L)	1.26±0.52	1.11±0.33	0.042

planus patients with/without HCV infections (Table 2).

A special analysis of differences related to TAC variations between the two groups of lichen planus patients was performed. Serum TAC levels reached statistically significant variations between lichen planus patients and negative HCV status and lichen planus patients and positive HCV status (1.26±0.52 mmol/L, 1.11±0.33 mmol/L, $p<0.05$).

Subsequently we analyzed the correlation between individual antioxidant level and serum total antioxidant capacity (Table 3). In lichen planus patients and negative HCV status the following statistical associations were identified: a weak positive borderline statistically significant association between albumin variations and TAC ($r=0.112$, $p=0.050$), a weak negative non-statistically significant correlation between blood iron levels and TAC ($r = –0.149$, $p>0.05$), between ferritin and TAC ($r = –0.103$, $p>0.05$), between ceruloplasmin and TAC ($r = –0.148$, $p>0.05$). In lichen planus patients and positive HCV status the following correlations were identified: a positive statistically significant association between albumin variations and TAC ($r = 0.301$, $p<0.050$), a moderate negative non-statistically significant association between blood copper levels and TAC ($r = –0.269$, $p>0.05$), and between ceruloplasmin and TAC ($r = –0.298$, $p>0.05$).

Table 3. Statistical correlations between TAC and different serum antioxidant levels

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

Discussions

Cells contain a complex network of antioxidant systems, which have the ability to prevent oxidative degradation of cell components (22). In recent years, findings show that there is a whole range of antioxidant factors at skin level, therefore the examination of the biological effects related to free radicals became a topic of increasing interest. Ferritin (in the cytoplasm), transferrin, lactoferrin, ceruloplasmin, albumin (in the extracellular fluid), vitamin E, ubiquinone, carotene (in the cell membrane), vitamin C (in the cytoplasm), glutathione (in the cytoplasm and mitochondrion), uric acid and bilirubin (in the blood), heme oxygenase-1 (in the dermis), heme oxygenase-2, catalase, superoxide dismutase (in the epidermis) (22) were described at skin level. It has been known that free radicals induce a decreased immune response (immunosuppression), leading to the impaired ability of the body to fight against different stimuli.

In this study, an analysis of the serum antioxidant status in active lichen planus patients was performed, aiming to identify the HCV infection-associated impact on disease exacer-

bation. Impairment of the TAC, which affected a significant proportion of the lichen planus patients versus control subjects, as well as the lichen planus patients and positive HCV status as compared to lichen planus patients and negative HCV status, may be a disease-associated condition.

Based on the results presented in this paper, we can identify two possible pathways which mediate TAC impairment in this disease.

First, the impairment of optimal iron and copper levels in lichen planus patients and positive HCV status leads to disruption of both cellular and systemic processes. The identification of a negative correlation between TAC and iron and TAC-ferritin, respectively, as well as between TAC and copper and TAC-ceruloplasmin, respectively, in lichen planus patients support the impact of these redox-active metals in the impairment of the cell redox potential. TAC provides information regarding the serum capacity to inactivate reactive radical species, by sequestration of free radicals and chelation of transition metals ions, thus preventing the Fenton reaction.

Second, the statistically significant positive correlation between TAC and albumin in lichen planus patients, particularly expressed in the HCV-associated disease, reveals the role of sulfhydryl groups in the impairment of the antioxidant potential in the extracellular space in these patients. TAC value may be a predictive factor of lichen planus recurrence.

The precise mechanisms enabling antioxidant systems to underlie disease exacerbations are not yet known, however specialists are constantly preoccupied with improved standardization of follow-up and diagnosis protocols in lichen planus patients. The authors believe that further studies designed to include the highest possible number of patients are needed to define the contribution of antioxidants to the understanding of lichen planus development. Determination of TAC may be an important outcome in the assessment of the association of lichen planus, HCV infection and the impaired antioxidant capacity of human serum. Literature provides little information regarding serum antioxidant status in lichen planus (1-9). Reduction of uric acid levels, inactivation of antioxidant enzymes, reduction of non-enzymatic antioxidants and impairment of the total antioxidant capacity support the appearance of an oxidants/antioxidants imbalance in lichen planus. Pathophysiological changes affecting the basal stratum, epithelial cells and the dermal-epidermal interface strengthen the association between oxidative stress and lichen planus development.

Conclusions

Based on these results, the authors deem lichen planus to be a complex medical condition, whose causes often remain unidentified and pathogenesis has not been cleared up. The potential involvement of several mutually enhancing mechanisms in the onset and progression of lichen planus is acceptable. Determination of serum non-enzymatic antioxidants may be useful in the establishment of the treatment approach and follow-up of lichen planus patients.

Bibliography

1. Georgescu SR, Ene CD, Nicolae I, Mitran M, Musetescu A, Clara Matei, Tampa M. Quantification of urine test strips through reflectometric analysis. Identification of various pathological conditions associated with lichen planus, *Materiale Plastice,* 2017 in press.
2. Park SY, Choi EH. Relevance of Herpes Simplex Virus Infection to Oral Lichen Planus, *Universal Journal of Medical Science* 2014; 2(3):25-30.
3. Gerayli S, Meshkat Z, Pasdar A et al. The Asssociation Between Oral Lichen Planus and Hepatitis C Virus Infection; A Report From Northeast of Iran. *Jundishapur Journal of Microbiology.* 2015; 8(4):e16741.
4. Krupaa RJ, Sankari SL, Masthan KMK, Rajesh E. Oral lichen planus: An overview. *J. Pharm. Bioallied. Sci.* 2015; 7(1):158-161.
5. Machin S E, McConnell D T, Adams JD, Vaginal lichen planus: preservation of sexual function in severe disease. BMJ Case Report. 2010; Published online. PMCID: PMC3029967

6. Gorouhi F, Davari P, Fazel N. Cutaneous and mucosal lichen planus: a comprehensive review of clinical subtypes, risk factors, diagnosis, and prognosis. *The Scientific World Journal*. 2014;2014.
7. Wagner G, Rose C, Sachse MM. Clinical variants of lichen planus. JDDG: *Journal der Deutschen Dermato-logischen Gesellschaft*. 2013;11(4):309-19.
8. Nogueira PA, Carneiro S, Ramos-e-Silva M. Oral lichen planus: an update on its pathogenesis. *International journal of dermatology*. 2015;54(9):1005-10.
9. Tampa M, Nicolae I, Ene CD, Sirbu I, Matei C, Georgescu SR. Vitamin C and TBARS in psoriasis vulgaris related to PASI, *Rev. Chim*. Buc. 2017;68(1):43-47 .
10. Battino M, Greabu M, Totan A, et al. Oxidative stress markers in oral lichen planus. *Biofactors*. 2008;33(4):301-10.
11. Chakraborti G, Biswas R, Chakraborti S, Sen PK. Altered serum uric Acid level in lichen planus patients. *Indian J Dermatol*. 2014 Nov;59(6):558-61.
12. Barikbin B, Yousefi M, Rahimi H, et al. Antioxidant status in patients with lichen planus. *Clin Exp Dermatol*. 2011;36(8):851-4.
13. Hassan I , Keen A, Majid S, Hassan T. Evaluation of the antioxidant status in patients of LPin Kashmir valley – A hospital based study. *Journal of Saudi Sociaty of Dermatology & Dermatologic Surgery*. 2013;17(1):13–16.
14. Azizi A, Farshchi F. Comparison of salivary and plasma antioxidant levels in lichen planus patients and healthy subjects. *J Oral Pathol Med*. 2012;41(7):524-6.
15. Abdolsamadi H, Rafieian N, Goodarzi MT, et al. Levels of salivary antioxidant vitamins and lipid peroxidation in patients with oral lichen planus and healthy individuals. *Chonnam Med J*. 2014;50(2):58-62..
16. Batu Ş, Ofluoğlu D, Ergun S, et al. Evaluation of prolidase activity and oxidative stress in patients with oral lichen planus and oral lichenoid contact reactions. *J Oral Pathol Med*. 2016;45(4):281-8.
17. Hashemy SI, Gharaei S, Vasigh S, et al. Oxidative stress factors and C-reactive protein in patients with oral lichen planus before and 2 weeks after treatment. *J Oral Pathol Med*. 2016;45(1):35-40.
18. Shiva A, Arab S. Evaluation of Uric Acid, Total Antioxidant and Lipid Peroxidation Parameters in Serum and Saliva of Patients with Oral Lichen Planus. *Global Journal of Health Science*. 2016;8(12):225.
19. Tavangar A, Ghalayani G, Alikhani M, Amrollahi N. Assessment of Salivary MDA and Antioxidant Vitamins in Patients with Erosive Type of Oral Lichen Planus and Lichenoid Reaction. OHDM. 2016;15(2).
20. Mishra SS, Maheswari TU. Evaluation of oxidative stress in oral lichen planus using malonaldehyde: A systematic review. *Journal of Dermatology & Dermatologic Surgery*. 2014;18(1):2-7.
21. Rahal A, Kumar A, Singh V et al. Oxidative stress, Prooxidant and Antioxidant:The Interplay, *BioMed Res. Int,* 2014; ID 761264:1-19
22. Georgescu SR, Ene CD, Tampa M, Matei C, Benea V, Nicolae I. Oxidative stress-related markers and alopecia areata. *Materiale Plastice* 2016; 53(3): 522-526.
23. Ene CD, Anghel AE, Neagu M, Nicolae I. 25-OH Vitamin D and Interleukin-8: Emerging Biomarker in Cutaneous Melanomas Development. Mediators of Inflammation 2015; ID904876.

Conflict of interest
NONE DECLARED

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