Reflectometric Analysis for Identification of Various Pathological Conditions Associated with Lichen Planus

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Reflectometric technique for analyzing urine strips represents a simple, fast and inexpensive method, with no risk and discomfort for the patient. In the present paper, the authors conducted a retrospective study on the possible association between lichen planus (LP) and a number of pathological conditions that produce changes of urine metabolites. A retrospective study was made on 77 patients with LP (49 cases of cutaneous LP - CLP, 28 cases of oral LP - OLP) who were diagnosed and treated in the dermatology department of Victor Babes Hospital for Infectious and Tropical Diseases. The quantification of certain urine metabolites in the spontaneous urine through reflectometric technique may reveal liver diseases (urobilinogen, bilirubin), kidney diseases (erythrocytes, proteins, albumin, creatinine, albumin/creatinine ratio, urine specific gravity, pH), metabolic disorders (glucose, ketones, pH, ascorbic acid) and urinary diseases (leucocyturia, nitrites, proteins, erythrocytes). The evaluation of the patients included clinical examination and laboratory and imaging tests. Among the investigated patients with LP we found: 16 cases of hepatic dysfunction (20.8%), 7 cases of renal dysfunction (9.1%), 11 cases of metabolic disorders (14.3%) and 14 cases of urinary tract infections (18.2%). No differences were found between patients with cutaneous and oral LP. The linear regression analysis revealed: positive association between concentrations of urobilinogen and bilirubin ($r = 0.174$, $p = 0.044$), negative association between albumin and creatinine ($r = -0.316$, $p = 0.007$), positive association between glucose and ketones ($r = 0.266$, $p = 0.098$) and positive association between leucocyturia and urine nitrite levels ($r = 0.202$, $p = 0.050$). Based on clinical and laboratory data, 85.7% of results obtained from urine biochemistry were confirmed. This analysis did not find any difference between patients with cutaneous and oral LP. By determining a full panel of urine metabolites through reflectometric analysis we were able to identify the main pathological changes in patients with LP.

Keywords: test strips, reflectance photometer, lichen planus

Lichen planus (LP) is a complex immunologically mediated disease, which affects the skin and mucous membranes, being an important concern for medical research [1-7]. The global prevalence of LP varies between 0.22 and 5% [8]. To this day its etiology is not fully understood. It seems that an autoimmune process develops and CD8 T lymphocytes have an important role leading to the apoptosis of basal keratinocytes [9]. Histological studies have revealed numerous CD8 T cells located in the dermis of LP lesions [10]. Moreover, an increased amount of IL 1beta, IL 4, IL-6, TNF-alpha and IFN gamma was observed. Cytokine production is involved in the recruitment of Langerhans cells and clonal expansion of cytotoxic T cells [10, 13].

The involvement of numerous factors has been mentioned in the pathogenesis of LP. It is thought that an exogenous antigen or an autoantigen, which triggers an inflammatory immune response is involved in the development of the lesions [14].The occurrence of LP can be caused by drugs (gold, lithium, methyldopa, quinine, quinidine, beta blockers, nonsteroidal anti-inflammatory drugs, diuretics, oral hypoglycemic agents, penicillamine, oral retroviral drugs). There have also been reported cases of LP which developed as a reaction to pre-existing skin lesions (herpes zoster, bruises, bites), after bone marrow transplant, stress, psychological trauma, or infections [1-3].

Oral lichen planus (OLP) may be associated with products used in dentistry, containing many potentially toxic allergenic substances. These include: metals and alloys (mercury, nickel, chromium, copper, silver, tin, gold, palladium, titanium, zirconium), resins, composites, acrylics, polymers, ceramics, adhesives, compounds used in dental procedures [3]. With respect to the dental materials used, the most cases of OLP have been reported to be associated to the use of amalgam, gold, and cobalt [15]. In the case of amalgam some authors believe that in fact there is a delayed hypersensitivity reaction to mercury, which is a component of the amalgam alloy and under certain conditions the amalgam alloy is deteriorated and its components including mercury are released [16].

In medical literature there are many reports about the association between LP and a number of pathological conditions: hepatic diseases (hepatitis B and C, chronic hepatitis, primary biliary cirrhosis), autoimmune diseases (ulcerative colitis, lupus erythematosus, vitiligo, alopecia areata, dermatomyositis, morphea, lichen sclerosus, myasthenia gravis), diabetes, cancer, arterial hypertension,

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infections (hepatitis C virus, herpes simplex virus), anxiety, urolithiasis, etc [1-6].

The reflectometric technique used to analyze urine strips is a fast method, useful in the identification of pathological conditions associated with LP, with no risk and discomfort for the patient. Various metabolites can be detected in urine samples in a wide spectrum of skin conditions [17,18]. In the present study, the authors conducted a retrospective analysis with regard to the possible association between LP and a number of changes that occur in certain pathological conditions using quantification of urine test strips through reflectometric analysis. To the best of our knowledge, this is the first study, which investigates the incidence of various pathological conditions in patients with CLP in comparison with OLP.

Experimental part

The present study was conducted with the consent of the Ethics Commission of the Victor Babes Hospital for Infectious and Tropical Diseases and the informed consent of the patient.

Study participants

We conducted a retrospective study on a sample of 77 patients with LP (fig. 1). The evaluation of the patients included clinical examination and laboratory and imaging tests.

Specimen: a spontaneous urine sample, preferably first morning urine collected in special tubes of 10 mL. The samples were processed immediately.

Method and materials

The reflectometric technique, a simple and inexpensive method, with no risk and discomfort for the patient is used to analyze urine test strips (urobilinogen, bilirubin, ketones, creatinine, red blood cells, proteins, albumin, nitrites, leukocytes, glucose, ascorbic acid, urine specific gravity, *p*H) (table 1). This test is fast and measures the urine elements which display significance for kidney, urinary, liver and metabolic diseases. In the event of a pathological condition, the strip color changes and the result is compared to a predefined color scale [15,16,19-21]. The color intensity allows a semi-quantitative assessment of the result (fig. 1). Additionally, the microscopic examination of urine samples was performed.

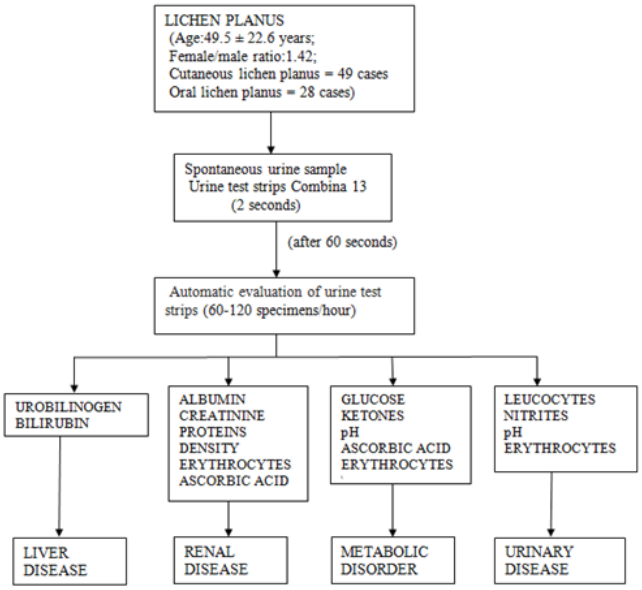

Fig. 1. Study design

Analyte	Reagent	Composition
Urobilinogen (mg/dl)	Diazonium salt	0.2%
Bilirubin (mg/dl)	Diazonium salt	0.6%
Ketones (mg/dl)	Sodium nitroprusside	5.7%
Creatinine (mg/dl)	3,5-Dinitrobenzoic acid	4.8%
Erythrocytes (cells/µl)	Diisopropylbenzene-dihydroperoxide Tetramethylbenzidine	26.0% 1.5%
Protein (mg/dl)	Tetrabromophenol blue	0.1%
Albumin (mg/l)	Sulfonephthalein	2.2%
Nitrite (mg/ml)	p-Arsanilic acid Tetrahydroquinoline	1.3% 0.9%
Leukocytes (cells/µl)	Pyrrole amino acid ester Diazonium salt	4.3% 0.4%
Glucose (mg/dl)	Glucose oxidase Peroxidase Potassium iodide	1.7% 0.2% 0.1%
Specific gravity	Poly (methyl vinyl ether/maleic anhydride) Bromothymol blue	90.2% 4.8%
pH	Bromocresol green Bromothymol blue	3.3% 55.0%
Ascorbic acid (mg/dl)	2,6-Dichlorophenolindophenol	0.8%

Table 1
REAGENT COMPOSITION FOR URINE TEST STRIPS

Statistical analysis

Comparison of quantitative variables for the two groups was performed by t test. It was chosen as a test of statistical significance 0.05 (5%), 95% confidence level showing that the decision is just. Correlations between variables were determined by linear regression and for the presentation of the relationship between two variables, Pearson correlation coefficient was used. Data processing was made using SPSS software.

Results and discussions

Urobilinogen (fig. 2). The test is based on the coupling reaction between diazonium salt and urobilinogen, the corresponding area of the strip turns pink. The color intensity corresponds to the following urobilinogen concentrations: 0.2, 1, 2, 4, 8 mg/dL. Under normal conditions, small amounts of urobilinogen, between 0.2 and 1.8 mg/dL are found in urine (table 2). Urobilinogen concentrations greater than 2.0 mg/dL are relevant to liver disorders.

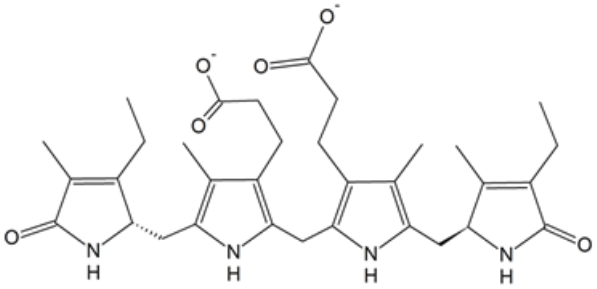

Fig. 2. Chemical structure of urobilinogen

Table 2

URINE UROBILINOGEN CONCENTRATIONS IN PATIENTS WITH LP

Urobilinogen (mg/dL)	CLP	OLP	Total
0.2-1	10	7	17
1-2	29	14	43
2-4	5	2	7
4-8	2	3	5
>8	3	2	5
Total	49	28	77

Bilirubin (fig. 3). Its detection is based on the coupling reaction between bilirubin and diazonium salt. The color intensity of the strip corresponds to the following bilirubin values: 0, 1, 3, 6 mg/dL. Bilirubin is absent in normal urine. Its presence in the urine is an early sign of liver dysfunction (table 3).

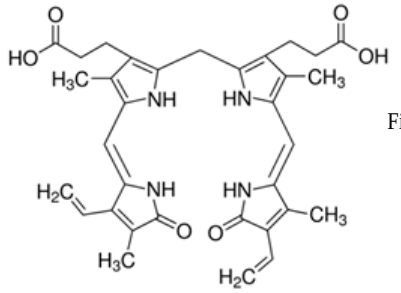

Fig. 3. Chemical structure of bilirubin

Table 3

URINE BILIRUBIN LEVELS IN PATIENTS WITH LP

Bilirubin (mg/dL)	CLP	OLP	Total
0-1	40	22	62
1-3	4	1	5
3-6	4	3	7
>6	1	1	2
Total	49	27	76

Ketones (fig. 4,5)

Their determination is based on the principle of Legal test: acetoacetic acid and acetone react with sodium nitroprusside, but beta-hydroxy butyric doesn't. The intensity of the resulting violet color allows the determination of the following ketone concentrations: 0, 5, 15, 39, 78, 160 mg/dL. Under normal conditions, ketones are absent in urine. The detection of ketones in urine is associated with altered cellular metabolism (table 4).

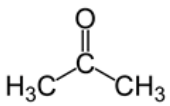

Fig. 4. Chemical structure of acetone

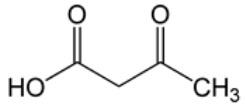

Fig. 5. Chemical structure of acetoacetic acid

Table 4

URINE KETONES LEVELS IN PATIENTS WITH LP

Ketones (mg/dL)	CLP	OLP	Total
0-5	41	25	66
5-15	2	0	2
15-39	5	2	7
39-78	1	1	2
Total	49	28	77

Creatinine (fig. 6). The test is based on the reaction of creatinine with 3,5-dihydroxybenzoic acid in an alkaline medium. Creatinine values correspond to the following concentrations: 10, 50, 100, 200, 300 mg/dL urine. Under normal conditions, increased amounts of creatinine are found in urine (200-300mg/dL urine). In the case of a kidney dysfunction, the amount of excreted creatinine decreases (table 5).

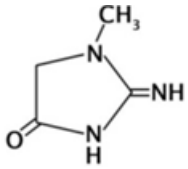

Fig. 6. Chemical structure of creatinine

Table 5

URINE CREATININE LEVELS IN PATIENTS WITH LP

Creatinine (mg/dL)	CLP	OLP	Total
10-50	2	1	3
50-100	3	2	5
100-200	41	17	58
200-300	3	8	11
Total	49	28	77

Erythrocytes

The test is based on pseudoperoxidase activity of hemoglobin and myoglobin which catalyze the oxidation of the indicator represented by diisopropyl-benzene dihydroperoxide and tetramethylbenzidine, resulting a green compound. The test allows the detection of the following values in the urine: 0, 10, 25, 80, 200 cells/μL. In normal urine, erythrocytes are absent, their presence is associated with kidney and urogenital tract diseases (table 6).

Table 6

ERYTHROCYTES IN URINE IN PATIENTS WITH LP

Erythrocytes (cells/μL)	CLP	OLP	Total
0-10	35	23	58
10-25	2	1	3
25-80	6	2	8
80-200	5	0	5
>200	1	2	3
Total	49	28	77

Proteins

The detection zone of proteins on the strip contains tetrabromophenol blue, which in the presence of proteins turns green, allowing the determination of the following concentrations: 0, 30, 100, 300, 2000 mg/dL. Under normal conditions, proteins are absent in urine. Presence of proteins in urine is the most important indicator of kidney disease (table 7).

Table 7

URINE PROTEIN LEVELS IN PATIENTS WITH LP

Proteins (mg/dL)	CLP	OLP	Total
0-30	26	12	38
30-100	12	7	19
100-300	8	7	15
300-2000	3	2	5
Total	49	28	77

Albumin

Albumin concentrations are proportional to the intensity of the color obtained in urine as the result of the reaction between albumin and sulfonephthalein and correspond to the following values: 10, 30, 80, 150 mg/L urine. In healthy individuals, very small amounts of albumin (below 20 mg/L) are detected in urine. In patients with renal disorders, abnormal loss of albumin is found (table 8).

Table 8

ALBUMINURIA LEVELS IN PATIENTS WITH LP

Albumin (mg/L)	CLP	OLP	Total
0-10	34	22	56
10-30	7	3	10
30-80	3	2	5
80-150	5	1	6
Total	49	28	77

Nitrite

The test is based on Griess reaction: nitrites react with p-arsanilic acid and tetrahydroquinoline, resulting a pink colored compound. The detection limit of the test is 0.1 mg/dL. In normal urine, nitrites are absent. The presence of nitrites in urine is associated with urinary tract infections caused by bacteria. It is estimated that 100,000 colony forming units (CFU) produce 0.075 mg nitrites/mL urine (table 9).

Table 9

URINE NITRITE LEVELS IN PATIENTS WITH LP

Nitrites (mg/dL)	CLP	OLP	Total
0-0.10	39	22	61
>0.10	10	6	16
Total	49	28	77

Leukocytes

The test is based on the reaction between granulocytic esterase and pyrrole amino acid ester/diazonium salt. If the corresponding area of the strip turns pink, the following values can be detected: 0, 15, 70, 125, 500 cells/μL. In normal urine, leukocytes are absent. The presence of leukocytes in urine is associated with inflammatory diseases of the urinary tract (table 10).

Glucose (fig. 7). The test is based on the reaction of D-glucose with glucose oxidase/peroxidase /potassium iodide. The colored complex formed in this reaction corresponds to the following glucose concentrations: 0, 50, 100, 250, 500, 1000 mg/dL. Under normal conditions the amount of urine glucose is under 50 mg/dL. Urine glucose level greater than 50 mg/dL is associated with important metabolic disorders (table 11).

Table 10

LEUKOCYTES IN URINE IN PATIENTS WITH LP

Leukocytes (cells/μl)	CLP	OLP	Total
0-15	33	24	57
15-70	2	2	4
70-125	6	2	8
125-500	8	0	8
Total	49	28	77

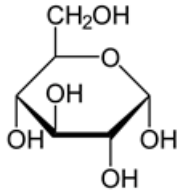

Fig. 7. Chemical structure of glucose

Table 11

URINE GLUCOSE LEVELS IN PATIENTS WITH LP

Glucose (mg/dL)	CLP	OLP	Total
0-50	27	23	50
50-100	15	3	18
100-250	4	1	5
250-500	3	2	5
Total	49	28	77

Urine specific gravity. The test is based on the reaction of ions in urine with poly (methyl vinyl ether/maleic anhydride)/bromothymol blue. The method allows the determination of the following values: 1.000, 1.005, 1.010, 1.015, 1.020, 1.025, 1.030. Normally, urine specific gravity is 1015-1025. Abnormal values of urine specific gravity indicate an impaired kidney ability to dilute or concentrate urine (table 12).

Table 12

URINE SPECIFIC GRAVITY LEVELS IN PATIENTS WITH LP

Urine specific gravity (mg/dL)	CLP	OLP	Total
1000-1015	18	11	29
1015-1025	22	14	36
>1.030	9	3	12
Total	49	28	77

pH

The test is based on bromocresol green/blue bromothymol capacity to change color intensity, depending on the *p*H value. The technique allows the identification of the following values: 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5. Normally, urine *p*H is 5.0-7.0. Abnormal urine *p*H is associated with systemic acid-base disorders of metabolic or respiratory origin (table 13).

Table 13

URINE *p*H lEVELS IN PATIENTS WITH LP

pH	CLP	OLP	Total
5-7	32	23	55
7-8	14	4	18
>8	3	1	4
Total	49	28	77

Ascorbic acid (fig. 8). The test is based on the reaction between ascorbic acid and 2,6-dichlorophenolindophenol. The decrease of chromogen intensity is proportional to the following concentrations: 0, 10, 25, 50, 100 mg/dL. Normal values are between 20 and 40 mg ascorbic acid/dl urine. Urine ascorbic acid variations are associated with renal dysfunction and altered cellular metabolism (table 14).

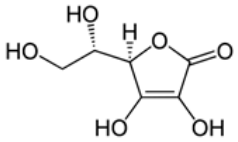

Fig. 8. Chemical structure of ascorbic acid

Table 14
URINE ASCORBIC ACID LEVELS IN PATIENTS WITH LP

Ascorbic acid (mg/dL)	CLP	OLP	Total
0-10	27	17	44
10-25	14	5	19
25-50	5	5	10
>50	3	1	4
Total	49	28	77

The quantification of certain metabolites in the spontaneous urine by reflectometric technique may reveal liver (urobilinogen, bilirubin), kidney (erythrocytes, protein, albumin, creatinine, albumin/creatinine ratio, urine specific gravity, *p*H), metabolic (glucose, ketones, *p*H, ascorbic acid) and urogenital disorders (leucocyturia, nitrites, protein, erythrocytes) (fig. 1). By analyzing urine strips the following results were obtained: liver dysfunction -16 cases (20.8%), renal dysfunction - 7 cases (9.1%), metabolic alterations - 11 cases (14.3%) and urinary tract infections - 14 cases (18.2%). No differences were found between patients with CLP and OLP (p = 0.127) (table 15).

Table 15
PREVALENCE OF PATHOLOGICAL CONDITIONS IDENTIFIED IN PATIENT WITH LP (p=0.127)

Disease	CLP (n=49)	OLP (n=28)
Liver diseases	10	6
Kidney diseases	5	2
Metabolic disorders	7	4
Urinary diseases	10	6

By linear regression analysis we found: positive association between concentrations of urobilinogen and bilirubin (r = 0.174, p = 0.044), negative association between albumin and creatinine (r = -0.316, p = 0.007), positive association between glucose and ketones (r = 0.266, p = 0.098) and positive association between leucocyturia and urine nitrite levels (r = 0.202, p = 0.050). Based on clinical and laboratory data, 85.7% of the results obtained from urine biochemistry were confirmed.

In our study, liver dysfunctions included chronic hepatitis with hepatitis viruses B and C, porphyria cutanea tarda and liver fibrosis. Many studies have reported contradictory associations between infections with hepatitis viruses and LP. The generation of these conflicting results could be caused by genetic susceptibility, host particularities, the diversity of geographical distribution of hepatitis infections, viral genotypes, immunological factors, the cytotoxic response of infected cells or the diversity of methods used [4].

Renal dysfunctions identified in our study, in patients with LP included diabetic nephropathy, lupus nephritis, membranous glomerulonephritis related to hepatitis B virus and renal lithiasis. There are few studies on the association between LP and kidney disease. In medical literature, urolithiasis is a common disorder encountered in patients with LP [5]. In this retrospective analysis metabolic disorders included type 2 diabetes, dyslipidemia, prolonged diarrhea and diabetic ketoacidosis. In a recent study, we have reported a series of metabolic disturbances in patients with LP, as hyperuricemia, hyperuricosuria, hyperoxaluria and hipocystatinuria [5]. In this study, urogenital abnormalities associated with LP, were represented by bacterial/fungal infections, urethritis, pyelonephritis, cystitis and problems in urine evacuation. Some studies have shown the association between vaginal LP and squamous cell carcinoma, human papilloma virus (HPV) infection or dysuria [6,7].

The authors mention that this paper is unique given the results discussed in the study: the frequency of pathological conditions associated with CLP versus OLP, the absence of noticeable differences regarding these pathologies in patients with CLP versus OLP, the use of a full panel of urine metabolites measured through reflectometric technique, the correlation of data obtained from urine analysis with information resulted from anamnesis, laboratory and imaging tests.

The limitations of this study are due to the low number of cases of LP included in the analysis. In further studies, the authors aim to investigate the ethiopatogenic mechanisms involved in the association between LP and certain systemic diseases.

Conclusions

Although the current analysis did not find any difference between patients with CLP and OLP, the determination of a full panel of urine metabolites through reflectometric technique allows a rapid and useful orientation with regard to the main pathological changes present in patients with LP.

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