Rīgas Stradiņa universitāte

Ilze Upeniece

INVESTIGATION OF PATHOGENETIC MECHANISMS IN VARIOUS CUTANEOUS *LICHEN PLANUS* CLINICAL MORPHOLOGICAL SUBTYPES

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ABBREVIATIONS

Abbreviation	English
AI	apoptotic index
CD4	cluster of differentiation 4
CD4+	cluster of differentiation 4 positive cell
CD8	cluster of differentiation 8
CD8+	cluster of differentiation 8 positive cell
CK15	cytokeratin 15
DC	dendritic cell
eHFSCs	epithelial hair follicle stem cells
IL	Interleukin
TNAEC	total number of apoptotic epithelial cells
TNEC	total number of epithelial cells
CB	Civatte bodies
LP	lichen planus
LPP	lichen planopilaris
LRP	lichen ruber planus
LC	Langerhans cell
MMP	matrix metalloproteinase
MMP-9	matrix metalloproteinases-9
MMPs	matrix metalloproteinases
NF-κB	nuclear factor-kB
S100	S100 calcium binding protein
S100+	S100 calcium binding protein positive cell
TGF-β	transforming growth factor β
TIMPs	tissue inhibitors of metalloproteinases
TNF-α	tumor necrosis factor α

Abbreviation	English
TUNEL	TdT-mediated dUTP nick-end labeling jeb terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate nick end-labeli
WS	Wickham striae

INTRODUCTION

Topicality of the research paper

Lichen planus (LP) is an acutely occurring inflammatory cutaneous disease frequently with a chronic course. It is characterized by erythematous to deeply purple lichenoid, planar, polygonal papules with thin, translucent and densely adherent scales. Lesions most commonly occur on the skin, oral mucosa and genital areas.

LP has a variety of clinical subtypes based on morphology: hypertrophic, hair and nails, atrophic, vesicular, pigmented, actinic, erosive, mucous etc. Each subtype has its own clinical and morphologic features (*Le Cleach et al., 2012*). Histological appearance basically is identical regardless of the localization involved and is characterized by hyperkeratosis, irregular "saw – tooth" acanthosis and wedge-shaped infiltration in the upper layers of dermis (*McKee, 1999*). Etiology of the dermatosis is unknown, but in the case reports (*Kazandijeva et al., 2007*) it has been shown, that LP arises in pigmented tattoos, autoimmune diseases (*Emad et al., 2012*), immunodeficiency (*Flamenbaum, 1982*), tumors (*Gibson and Murphy, 1997*), and other conditions, but mostly in the case of chronic hepatitis C (*Sayiner et al., 2017*).

Pathogenesis of LP is uncertain (*Le Cleach et al., 2012*). Dense infiltrate of T – lymphocytes in the papillary layer of dermis can induce apoptosis of basal keratinocytes (*Hussein, 2007*) and is known as one of the most characteristic features of LP. $CD4^+$ and $CD8^+$ T – lymphocytes can be found in the infiltrate of LP, and CD8+ is predominant in lesions (*Wolff et al., 2016*).

Hair follicle alternating lifelong restructuring activity proves the presence of its own stem cells (*Paus and Cotsarelis, 1999*), called epithelial hair follicle stem cells located in the hair follicle bulge region, at the insertion site of the arrector pili muscle in the outermost layer of the outer root sheath

(*Bardazzi et al., 1999*). *Mobini et al.* (2005) expressed an opinion, that lesion of epithelial hair follicle stem cells, characterized by reduced or absent CK15 immunosuppression, which is possibly caused by $CD8^+$ cytotoxic cells, plays role in pathogenesis of primary cicatricial alopecia, comprising one of LP subtypes (*Mobini et al., 2005*).

It is known, that $S100^+$ cells can be found in the skin affected by the disease (*Eckert et al., 2004*), and the distribution in epidermis varies (*Broome et al., 2003; Schonthaler et al., 2013*). $S100^+$ cells are dendritic cells (DC), amount of which is significantly increased in case of LP (*Lee et al., 1996*).

It is possible that in case of LP there are several cell death mechanisms. Keratinocyte death, especially in the basal layer, induces $CD8^+$ cytotoxic T lymphocytes and natural killer cells (*Sugerman et al., 2002; Drogoszewska et al., 2014; Gaber et al., 2014*). Cell death can be induced by other mechanisms as well, without the presence of T cells, for example, disruption of cell-to-cell, cell-to-matrix interactions (*Kastelan and Massari et al., 2007; Ernst et al., 2013*).

Matrix metalloproteinases (MMPs) are important cell enzymes. MMP-9 is gelatinase that splits denatured collagen and gelatine molecules (*Visse and Nagasse, 2003*). It is closely related to malignization process (*Patel, 2007*), promotes apoptosis (*Gunduz et al., 2006*), causes basement membrane (BM) rupture (*Zhou et al., 2001*), although, in case of dermal LP the risk of malignization is smaller than 1% (*Sigurgeirsson and Lindelof, 1991*).

Dermatoscopy is a non-invasive method of visualization, extensively used in diagnostics of cutaneous tumours. It is also used in cases of skin infection, for evaluation of hair and nail structure and visualization of blood vessels. Recommendations for dermatoscopic diagnostics could be retrived from literature, but these are far from being complete (*Lallas et al., 2012*) and it has no certain clinical, pathogenetic or prognostic meaning.

Available ultrastructural LP data is poor. Analysis of clinical cases demonstrates damage in cell junctions and the basement membrane (BM) rupture (*Hirota and Osaki, 1992*).

Necessity to understand the skin structure changes in various clinical and morphologic cutaneous LP cases, to correlate them with application of contemporary instrumental methods of modern dermatology clinic, to expand our knowledge about LP pathogenesis, points out the significance of the chosen theme. More information on pathogenesis of the disease can be obtained by a set of multiple, simultaneously used methods, including light microscopy, electron microscopy, immunohistochemical methods and skin damage surface visualization method – digital dermatoscopy.

Aim of the study

Clinical, dermatoscopic and morphologic description of cutaneous *lichen planus*, determining traits applicable to the activity of the disease; morpho-functional evaluation of the tissue material to deepen our knowledge of the pathogenesis of disease.

Study objectives

To achieve the aim of the promotion thesis, the following objectives were set:

1. To gather the demographical and clinical data on *lichen planus* patients.
2. To make a digital dermatoscopy of the cutaneous lesions and evaluate their dermatoscopic parameters: blood vessels morphology, background colour, pigmentation and presence of Wickham striae.

3. To evaluate the structural changes of skin on cellular and tissue level in various subtypes of cutaneous *lichen planus* by using routine staining and histochemical methods.
4. To study and estimate semiquantitatively epidermal and follicular expression of CK15 in *lichen planus* tissues.
5. To study distribution of cutaneous S100 positive cells in *lichen planus* lesions evaluating the involvement of immune cells in the pathogenesis of LP.
6. To study enzymatic degradation of extracellular matrix components in *lichen planus* lesions and its involvement in the pathogenesis of disease evaluating MMP-9 expression.
7. To study peculiarities of cell death in *lichen planus* tissues by using TUNEL reaction and estimating the apoptotic index within the epidermis, as well as correlating these data with ultrastructural changes of cytoskeleton and the basement membrane region characteristic of keratinocytes apoptosis.
8. To analyze clinical course of *lichen planus* correlating indicators of disease progression and prognosis with biopsy histopathology and dermatoscopy findings.

Hypothesis

1. The subtypes and disease activity of cutaneous *lichen planus* are characterized by certain clinical, dermatoscopic and morphologic picture.
2. Cell differentiation and death, extracellular matrix remodeling and involvement of immune system cells play a role in the pathogenesis of cutaneous *lichen planus*.

Novelty of the Thesis

In the thesis a compilation of clinical data from patients affected by LP, during a particular period of time in a single dermatology clinic in Latvia, was made. In this study, paralell to clinical and instrumental data analysis, for the first time investigation of the tissues affected by cutaneous *lichen planus* was made, using various morphological methods simultaneously – histochemical, immunohistochemical (MMP-9, CK15, S100), TUNEL method and electron microscopy. Dermatoscopy performed aimed diagnostic and prognostic purpose. Until now, such data is described seperately in the world literature, but no complex data is published.

Target population

Target population is Riga 1st Hospital Clinical Centre of Skin and Sexually-Transmitted Diseases registered patients with cutaneous *lichen planus* during the period from 2008 to 2014. For positive control, eight psoriasis patients' tissue material was retrieved from archived skin biopsy paraffin blocks in Riga 1st Hospital Clinical Centre of Skin and Sexually-Transmitted Diseases. For negative control, material from eleven volunteers, without inflammatory dermatosis and visible changes on the skin, was used.

Cooperation partners

1. Riga 1st Hospital Clinical Centre of Skin and Sexually-Transmitted Diseases (dr. M. Skudra).
2. RSU Department of physics (lecturer V. Cauce).

Material technical supply

Material technical resources were used for this research project, using the rescourses of RSU AAI joint Laboratory of Electron Microscopy and Riga 1st Hospital Clinical Centre of Skin and Sexually-Transmitted Diseases, as well as the opportunities provided by RSU doctors grant.

The Author's Personal Contribution

The author of the thesis has designed the study, conducted literature analysis, selected archived tissue material, compiled patient's clinical data from medical records, recruited patients and performed the skin biopsy, performed immunohistochemical reactions and TUNEL reaction, analyzed the tissue changes by using light and electron microscopy, conducted dermatoscopic analysis of the cutaneous lesions, photography and statistical data analysis..

Ethical aspects

Permission to conduct this research was received from The Ethics Committee of Rīga Stradiņš University on 27th of September 2012.

1. LITERATURE REVIEW

1.1. Epidemiology of *lichen planus*

LP is a chronic inflammatory and immune mediated disease that affects the skin, nails, hair and mucous membranes. LP belongs to papulosquamous dermatoses, the primary etiology of which is unknown *(Fox and Odom*, 1985). The exact prevalence of LP is unknown. Cutaneous LP in adults is present in 0,2-1% of population (*Shiohara and Kano*, 2012).

1.2. The clinical manifestations of *lichen planus*

Primary skin damage is itchy, various shape and size, red/purple coloured papules or nodes (*Le Cleach and Chosidow*, 2012). The skin damage can appear on any location, but it is most common on the skin of palms, back, extremities and genital area (*McCall and Lawley*, 2008). Cutaneous LP has several different clinically relevant morphologic subtypes, based on the site of (I) involvement, (II) configuration and (III) histopathological findings: (I) palms and soles, mucous membranes, nails, scalp, inverse, erythrodermic; (II) annular, linear; (III) papular or classic LP (*lichen ruber planus* (LRP)), hypertrophic, atrophic, vesiculobullous, erosive/ulcerative, follicular (*lichen planopilaris* (LPP)), actinic, LP pigmentosus, perforating.

1.3. General morphological characteristics of skin affected by *lichen planus*

Signs of the classic histopathological LP include the presence of dense, band-like infiltrate of lymphocytes and histiocytes at the dermal-epidermal junction, hyperkeratosis or orthokeratosis, hyper-granulosis, irregular

"saw-tooth" acanthosis, basal cell vacuolar degeneration, rete ridges spongiosis and apoptosis of keratinocytes (*Gorouhi et al*., 2014).

1.4. Dermatoscopic characteristics of *lichen planus*

Dermatoscopy allows visualization of WS, which is considered as a sensitive and specific criterion for LP diagnosis. WS has been observed as a round, linear, square, or ring-shaped white structures, which may produce both fine and wide arboriform projections, which can be seen with dotted or linear blood vessels around them marking out the projections (*Lallas et al*., 2014). Dermatoscopically, two different types of LP hyperpigmentation can be distinguished: a brownish dispersed form, possibly associated with epidermal pigmentation, and a deep, granular type, which, in turn, corresponds to the pigmentation in the dermal melanophages (*Vázquez-López et al*., 2003).

1.5. The pathogenesis of *lichen planus*

Specific mechanisms are involved in the pathogenesis of the disease, for example, antigen presentation, activation of T lymphocytes, cell death, and nonspecific mechanisms, for example, mast cell degranulation, activation of metalloproteinases (*Sugerman et al*., 2002). The disease has a chronic and relapsing course, which is partially explained by the involvement of transforming growth factor-beta (TGF-β) (*Gorsky et al*., 2004). Cytokines involved include interferon-γ (IF-γ), tumor necrosis factor-α (TNF-α) (*Sugerman et al*., 2002), nuclear factor-kB (NF-κB) dependent cytokines, for instance, interleukin-1α (IL-1α), IL-6 and IL-8, as well as other apoptosis-related molecules (*Lehman et al*., 2009).

1.5.1. A role of cytotoxic T lymphocytes in the pathogenesis *of lichen planus*

CD4+ and CD8+ T lymphocytes can be found in the infiltrate of LP, CD8+ is predominant in lesions (*Wolff et al*., 2016). It has been shown, that the activated T-lymphocytes can induce basal keratinocyte apoptosis (*Hussein*, 2007). Schematic representation of LP pathogenesis can be viewed in the figure 1.1.

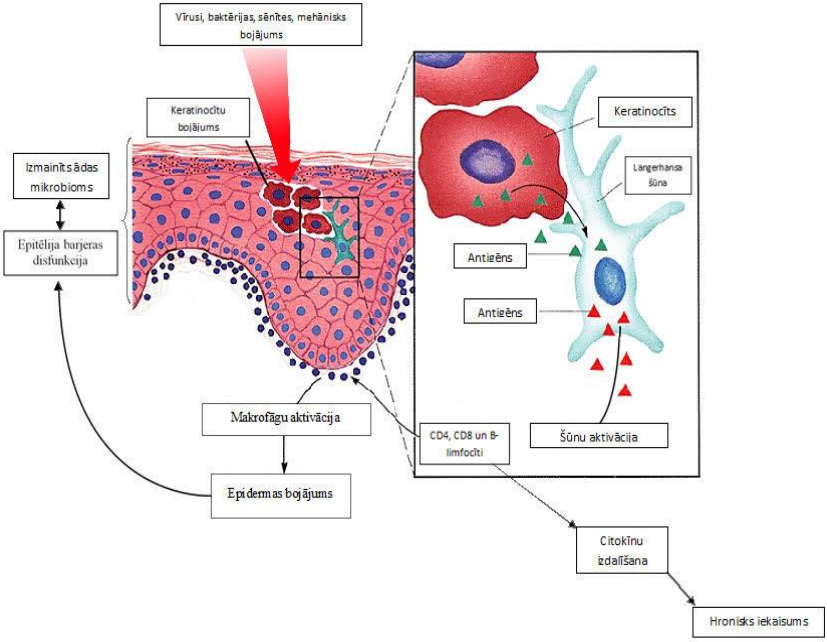

Figure 1.1. **Pathogenesis of the LP***

*Modified by *Rubin et al*., 2008; *Baek and Choi*, 2017

1.5.2. The importance of dendritic cells in the pathogenesis of *lichen planus*

S100 proteins are expressed in normal and LP affected skin (*Eckert et al*., 2004; *Santoro et al*., 2005), and their subcellular distribution in keratinocytes is varying (*Broome et al*., 2003; *Schonthaler et al*., 2013). S100+ are also dendritic cells (DC), that are involved in antigen presentations, healing and repair processes, and the amount of which is much bigger in the case of LP (*Lee et al*., 1996).

1.5.3. The importance of cytokeratin 15 recognition in the pathogenesis of *lichen planus*

It is stated, that epithelial hair follicle stem cells (eHFSCs) are located in the bulge region, at the insertion site of the arrector pili muscle in the outermost layer of the outer root sheath (*Harries and Paus*, 2010), and contribute to regeneration of the epidermis after damage (*Paus and Cotsarelis*, 1999). According to results, published by several leading researchers (*Sabeti et al*., 2013; *Abbas and Bhawan*, 2011; *Abbas and Mahalingam*, 2009; *Kloepper et al*., 2008), presence of cytokeratin 15 (CK15), stem cell marker, labels the cells of the human hair follicle in the bulge region, the outermost layer of the outer root sheath, the basal layer of the epidermis (see Fig. 1.2.) and eccrine glands. Furthermore, inflammatory cell infiltrate in the bulge region is likely to have a strong impact on hair follicle stem cell deficiency in LPP cases, that explains permanent hair loss (cicatricial alopecia), occurring in LPP (*Al-Refu*, 2012).

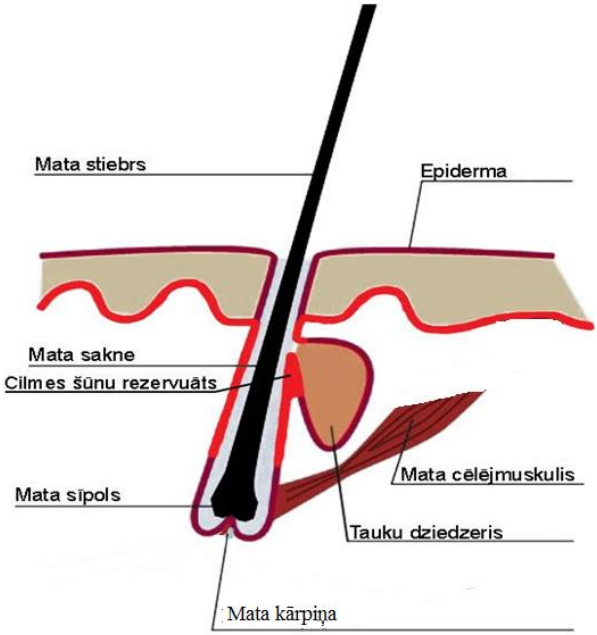

Figure 1.2. **Schematic representation of CK 15 expressing cells**

The expression of CK15
* Modified by *Hoang et al*., 2009

1.5.4. The relevance of matrix metalloproteinase-9 in the pathogenesis of *lichen planus*

Matrix metalloproteinases (MMPs) belong to the zinc-dependent endopeptidases family, capable to perform remodelling and degradation of extracellular matrix components. *Zhou* with a working group (2001) reported, that T-cells from oral LP lesions secretes more MMP-9 than controls, and expressed a thought, that MMP-9 created by the T cells may be crucial in the pathogenesis of LP, leading to ruptures of BM and contributing to apoptosis (*Zhou et al*., 2001).

1.5.5. The importance of programmed cell death or apoptosis in the pathogenesis of *lichen planus*

In the case of LP, the process of apoptosis, caused by CD8+ cytotoxic lymphocytes and natural killer cells, follows either perforin/granzyme pathway or Fas/Fas ligand path, when cytotoxic proteins are discharged to trigger the death of the disseminated keratinocytes (*Drogoszewska et al*., 2014; *Saleh et al*., 2014; *Gaber et al*., 2014; *Su and Chung*, 2014; *Sugerman et al*., 2002; *Neppelberg et al*., 2001). However, in case of this disease, cell death, which is observed even without the presence of T-cells, can be described by loss of cell-to-cell, cell-to-matrix contacts (*Ernst et al*., 2013; *Neppelberg et al*., 2001).

2. MATERIAL AND METHODS

2.1. Study material and patients groups

2.1.1. *Lichen planus* patient selection

The study is combined in time. The retrospective phase included data collection from patient's medical records during the period from 2008 to 2012. The prospective phase of the study covered the period from 2012 to 2014. Patients of Riga 1st Hospital Clinical Centre of Skin and Sexually-Transmitted Diseases with clinical and morphologic diagnosis of LP were enrolled in this study. Altogether 6 patient groups were created: (I) LRP, (II) hypertrophic LP, (III) atrophic LP, (IV) vesiculobullous LP, (V) LP pigmentosus and (VI) LPP. Follicle or LPP was subdivided based on the localization of lesions: a) corpus LPP and b) scalp LPP.

2.1.2. Control groups

To increase the objectivization and reliability of the rstudy results, two control groups were created – positive and negative. For the positive control material obtained from eight patients, whose diagnosis was plaque psoriasis or vulgar psoriasis (*Psoriasis vulgaris*) was used. Eleven volunteers with no inflammatory skin disease history and existing visual changes in the skin were used for negative control. The skin tissue material of this control group was obtained by performing a skin biopsy at the Riga 1st Hospital Clinical Centre of Skin and STD.

2.2. Research methods

2.2.1. Collection and summarization of clinical data

One hundred and seventeen patients (43 – men and 74 – women) aged from 16 to 89 years, whose diagnosis is cutaneous LP, were included in the study. Patient demographic data, including gender and age, was collected. Clinical data included complaints of skin rash, cutaneous LP duration in months and years, localization, rash characteristics. Patient topographic maps were constructed for characterization of skin rash localization. The clinical picture is photodocumented in the first appointment, using a digital camera (Nikon Coolpix P500), previously eliminating any identification features of a natural person (jewelry, tattoos, piercings, profile image).

2.2.2. Dermatoscopic examination

Primary rash (2 pieces) of the patients was examined, using a digital dermatoscope microDERM® (Visiomed, Germany), prior to the initiation of therapy. Dermatoscopic image was photodocumented, using digital dermatoscope system (original zoom ×15, ×30 or ×50). In order to ensure the best image quality available, minimal pressure was used and skin rash surface was covered in oil or water during the examination. Dermatoscopic evaluation was performed by one doctor (Ilze Upeniece - author of the thesis). The dermatoscopic evaluation includes the following parameters: (I) vascular morphology (dotted, linear, dotted + linear), (II) arrangement of blood vessels (regular, spotted, peripheral); (III) skin background colour (red, i.e. bright red color, light red color, i.e. volatile red, yellowish); (IV) the presence of streaky fine white lines (Wickham striae) and (V) the presence of hyperpigmentation (superficial dispersed pigmentation, localized granular pigmentation).

2.2.3. Performance of skin biopsy

In this study, archived skin biopsy material was obtained by skin punchers ranged from 1.5 mm to 3 mm in diameter. In the prospective part of the study, two skin biopsies per patient were performed and the tissues were further processed for morphology analysis by using a light and electron microscopy. The skin biopsy was performed using Punch technique.

2.2.4. Histochemical staining methods

The punch biopsy samples were prepared according to literature recommendations (*Celis, 1994*). The hematoxylin and eosin staining was necessary to perform the routine morphological analysis (*Gorouhi et al*., 2014; *Wolff et al*., 2016). The structural changes of the skin were analyzed according to *Olsen et al*. guidelines (2003) and *Tandor et al*. Recommendations (*Olsen et al*., 2003; *Tandon et al*., 2008). *Verhoeff-Van Gieson* method and staining with toluidine blue was applied to better assess the dermal connective tissue – elastic, collagenous placement and amount, for example, in cases of fibrosis.

2.2.5. Immunohistochemistry methods

The immunohistochemistry method was necessary to determine the presence of the antigen and its location in the patient's tissue (*Celis*, 1994). Following the recommendations of the manufacturers, three different visualization systems were used in the study. The antigens used for the immunohistochemistry method are S100, CK15 and MMP-9. Immunohistochemical reactions were evaluated both quantitatively and semi-quantitatively.

2.2.6. TUNEL method

In order to detect the programmed cell death or apoptosis *terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling* (TUNEL) method is used. The evaluation of apoptosis marker expression was performed by determining apoptotic index (AI) (*Brant et al*., 2008).

2.2.7. Transmission electron microscopy method

Skin biopsy material for electron microscopic analysis was cut into 1 mm^3 pieces, from that a material was prepared to be embedded in epoxy resin, according to *Celis* recommendations (*Celis*, 1994).

From epoxy resin embedded tissue material, using ultramicrotome (Y2088, LKB, Sweden), 1-2 nm thick sections or semithin sections and 60-80 nm thick or ultrathin sections were cut.

Tissue analysis was launched in electron microscope (JEOL 1011 transmission electron microscope, Japan) magnification ×2000 – ×3000, then gradually a higher magnification up to ×40000 was chosen. The cell sections useful for the objective of the study were analysed described and, if necessary, photographed with Kodak image plates (SO-163, Kodak, *Rochester, N.Y.*).

2.2.8. Statistical analysis of the data

Quantitative variables were described by the arithmetic average and standard deviations (SD) as well as medians with interquartile range (IQR). Categorical parameters were expressed as frequencies and percentages, after being submitted to a Kolmogorov-Smirnov test to detect any differences

between samples. Dispersion analysis (ANOVA) was performed using *Post Hoc Test* with *Bonferroni* correction.

For localization of different groups into couples *Wilcoxon Signed Ranks, Friedman,* and *Chi-Square* tests were used. Values of $p < 0.05$were considered as significant.SPSS 21.0 version program was used for statistic analysis.

3. RESULTS

3.1. Results of demographic data and clinical examinations of *lichen planus* patients

117 patients in age between 16 and 89 were included in the study. The average age of the patients was 51.9 (SD 18.9) years. 43 men (36.8%) and 74 women (63.2 %) were included in the study. The average age of the men was 42.4 (SD 17.4) years, the minimal age was 16 years and the maximal was 88 years. The average age of women was 57.6 (SD 17.6) years, minimal 16 years, maximal 89 years. The average age of men – 42.4 (SD 17.4) years significantly differed from the average age of the women – 57.6 (SD 17.6) years ($p < 0.001$) (see Fig. 3.1.).

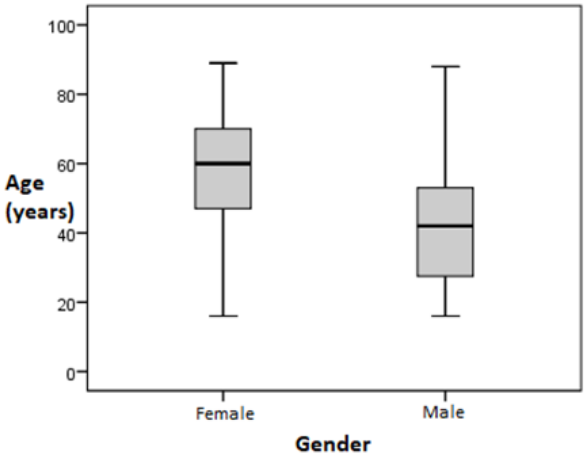

Figure 3.1. **Age of patients**

The largest group for men is 31-60 years (46.3 %, n = 19) and the largest group for women is >60 years (47.9 %, n = 34). These differences are statistically significant ($p = 0.001$).

Most commonly polygonic, cyanotically pink, glossy papules on the skin of head, corpus and extremities were observed. In many places of the surface of the elements flaky, densely adhered, white scale was observed. Quite often haemorrhagic dotted scab was observed, which indicated to presence of irritation. During regression stages of the disease brown hyperpigmented spots were observed (see Fig. 3.2.).

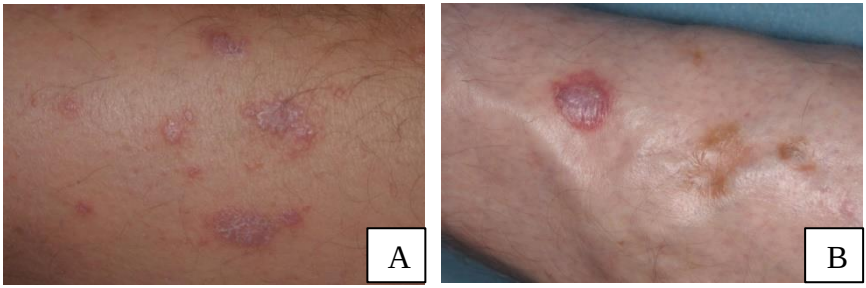

Figure 3.2. **LP clinical presentation**

A) Polygonal, flat-topped, purple papules. *Wickham striae* – white netting on the surface of papule; B) In the skin of the lower leg LP papule with densely adherent scales and hyperpigmented brown spots corresponding to the location of the former LP primary elements

3.2. Results of dermatoscopic examination

Digital dermatoscopic examination was carried out on 13 patients and two LP elements were analysed in different localizations of the skin (see Fig. 3.3.). In the prospective part of the study 9 women and 4 men were included. All together 26 LP elements evidenced in cases with established morphological diagnosis of LRP were analysed.

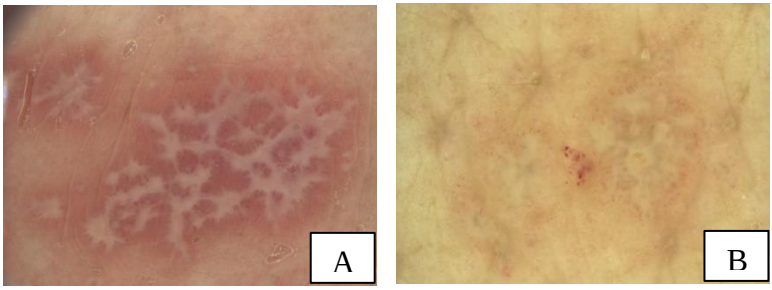

Figure 3.3. **LP dermatoscopic depiction**

A) On a red background, white cross-linked lines, or the *Wickham striae*, dotted and linear blood vessels with peripheral placement; B) Skin damage in the regression phase – a yellowish background with dotted blood vessels and *Wickham striae*, diffused pigmentation

Dotted + linear blood vessels were an often manifestation of LP (53.8 %). *Wickham striae* was observed in most of LP elements (73.1 %). It was spotted that in the elements in which *Wickham striae* was absent, location of the blood vessels was even or spotty, whereas all elements with peripheral position of the blood vessels (50 %, n=13), *Wickham striae* was present (p=0.003). In 15 elements of LP dissipated light brown homogeneous pigmentation was observed, whereas localized granule-type pigmentation was spotted in 8 LP elements. 9 LP elements contained both types of pigmentation.

3.3. Results of routine and histochemical staining analysis

During the study period 6 of 9 LP clinically morphological subtypes were diagnosed: LRP, vezikulobullose, pigmental, hypertrophycal, atrophycal and LPP. The most frequent subtype of LP was LRP (43.6 %, n = 51). The most infequent subtype of LP was pigmental (0.9 %, n = 1). The distribution of amount of patients according to clinically morphological subtypes of LP is summarized in figure 3.4.

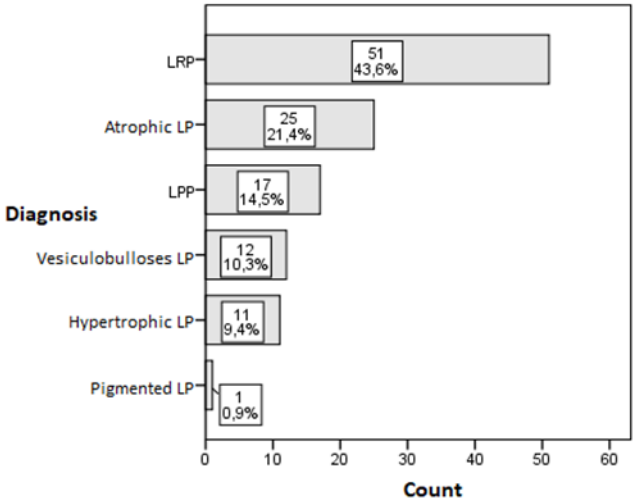

Figure 3.4. **The distribution of amount of patients accordding to clinically morphological subtypes of LP**

The youngest popullation was observed in case of hypertrophic LP, but the eldest in case of atrophic and vezikulobullose LP, where accordingly the average age was 43.7 (SD 16.2), 61.1 (SD 19.4) and 60.1 (SD 16.1) years.

The plurality of male gender was observed in the case of LRP, hypertrophic and vesiculobullous LP (46.5 %, 16.3 %, 11.6 %), unlike other LP subtypes.

92 (78.6%) cases were localized, but the disseminated form was 25 (21.4%) cases. Skin itching was observed in 100 (85.5 %) cases. There are no statistically significant differences between localization of the process or dissemination and itching (p = 0.115). The most frequent localization of LP was leg skin (63.3 %, n = 74), hands (53 %, n = 62), and corpus slightly less (41.2 %, n = 47), and the less common site was head (14.5 %, n = 17). In general, the most commonly observed localization of the LP was legs (39.9 %)

and hands (36.2%), but the least common site was torso (13.5 %) and head (10.4%), according to the total number of patients.

The most commonly seen in the arms and legs was LRP (59.3 %, 55.4%), and the most commonly in the torso skin the atrophic LP (36.4 %). In contrast, LPP is the most common LP subtype in the scalp (64.7 %). The frequency of affected skin regions in relation to LP subtypes is summarized in the figure 3.5. The skin of the torso was not characterized by hypertrophic LP and the head skin with hypertrophic and vesiculobullous LP (0). In the head skin, the most frequent rash was women in relation to men (88.2 %, n = 15, 11.8 %, n = 2). For men, the most frequent localization of LP was leg skin (43.1 %, n = 28).

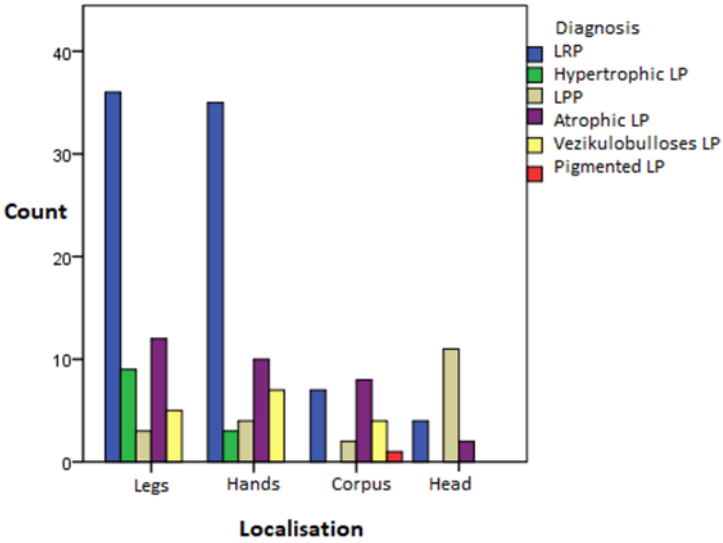

Figure 3.5. **Incidence of affected skin areas according to LP subtypes**

In histological preparations, LP is characterized by compact orthoceratosis, irregular acanthosis and vacuolation of basal cells. In the upper layer of the dermis, band-like lymphocyte inflammation was observed accompanied by accasional eosinophilic leukocytes (See Fig. 3.6.).

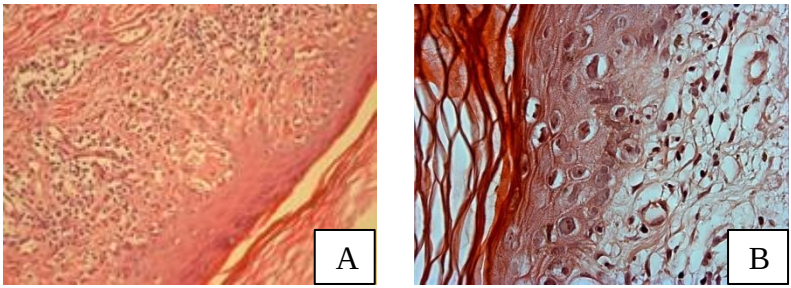

Figure 3.6. **LP histopathological presentation**

A) irregular "saw – tooth" acanthosis, basal cell vacuolization. In the upper layer of dermis, a band-like lymphocyte inflammation with separate occasional melanophages, hematoxylin and eosin, × 250; B) Affection of keratinocytes' intercellular contacts, hematoxylin and eosin, × 400

In the fresh rash, the infiltration that penetrated the epidermis, causing irregular acanthosis, prevailed. The melanophages were quite often seen. In the old rash, the infiltration density decreased, but the number of melanophages increased. In several cases, homogeneous eosinophilic structures, which were keratinocyte remnants or colloidal bodies, were observed in the basal layer of the epidermis and dermis.

In the hypertrophic LP variant, we determined significant changes in the epidermis – acanthosis, hypergranulosis and hyperkeratosis; in the atrophic LP variant, epidermal atrophy, flattened warts, a slight hypergranulation, perivascular infiltration, and a large number of melanophages. In the case of a follicular LP variant, we observed a hair ostium filled with a thick horn cork, follicular sheath lichenoid damage, vacuolation and spongiosis. Separation of the epidermis from the dermis was sometimes observable with the *Max-Joseph* gap. These changes were characterized by the vesiculobullous variant of LP.

Correlations between greatly varying cutaneous rash elements, intensity of inflammatory infiltrates, cellular composition and distribution of blood

vessel have been shown. Differences in the number and spacing of elastic and collagen fibres, as well as the presence of dermis fibrosis, were studied using the *Verhoeff-Van Gieson* histochemical method (see Fig. 3.7.).

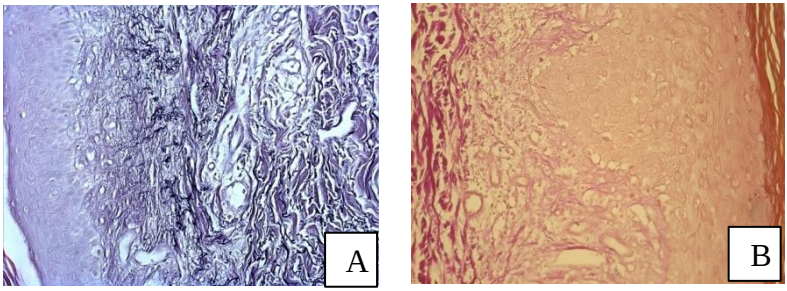

Figure 3.7. **A) LP microphotography: the morphological depiction of elastic fibers**

Verhoeff-Van Gieson, × 250

B) LP microphotography: the appearance of collagen fibers

Loose collagen fibers in the papillary dermis and in the form of bundles in the reticular dermis,

Verhoeff-Van Gieson, × 200

3.4. Immunohistochemical analysis of skin dendritic cells

S100 antigen revealed cytoplasmic as well as nuclear expression confirmed in all study groups (see Fig. 3.8.). In the epidermis, $S100^+$ cells were observed in the spinous layer, also in suprabazal and basal localization. In contrast, in the dermis $S100^+$ cells are subepidermal.

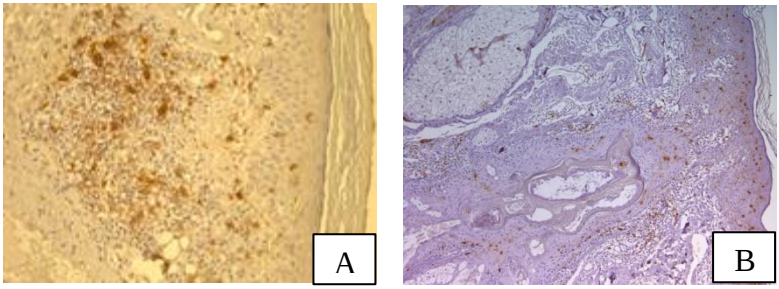

Figure 3.8. **A) LP microphotography: S100+ cells in the epidermis and subepidermal inflammatory infiltration**

Hypertrophic LP, high number of S100+ cells in the dermal inflammatory infiltrate, × 250

B) LP Microphotography: S100 + cells in the case of LPP

High number of S100+ cells in the epidermis and outer epithelial root sheath in the scalp LPP, × 100

In the hair follicle, $S100^+$ cells were observed in the outer epithelial roots sheath. In the eccrine and apocrine sweat glands and sebaceous glands, $S100^+$ cells were not observed. Most $S100^+$ dermal cells were perivascular. In LPP patients, the scalp region showed a more pronounced and denser S100 immunoreactivity when compared to the corpus. However, in the quantitative assessment there was no difference between S100 positive cells in the epidermis and the hair follicle by analysing samples from the LRP and LPP patient groups: body LPP 9.0 (IQR 6; 11.0), scalp area 4.0 (IQR 2.3, 18.8) and LRP 10.5 (IQR 6.0; 14.3). The immunolabeling of the epidermis S100 in psoriasis samples was virtually nil – 0.0 (IQR 0.0; 1.0) and statistically significantly different from the previous groups ($p < 0.001$). The mean number of $S100^+$ cells determined in the subepidermal region was statistically significantly higher in the LRP samples than in the LPP group ($p < 0.05$).

3.5. Immunohistochemical analysis of cytokeratin 15 expression

Cells labeled by the anti-CK15 antibody displayed a brown cytoplasmic staining pattern. Expression of CK15 in epithelial cells was demonstrated in the outer and inner root sheath of hair follicles (see Fig. 3.9.), the basal layer of epidermis and eccrine glands. The results describing levels of epidermal and follicular cytokeratin expression and found to be greatly varying from absence and weak to strong are summarized in Fig. 3.10.

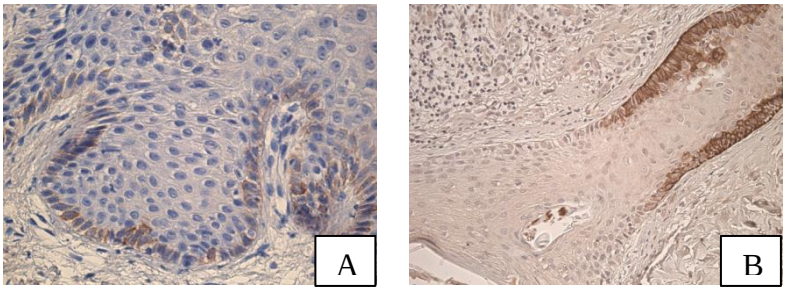

Figure 3.9. **A) LP microphotography: expression of CK15 in the epidermis, × 400**
B) LP Microphotography: Expression of CK15 in case of LPP
Strong (+++) expression in the outer root sheath of hair follicle and negative (-) to weak (+) expression near the infiltrate, × 250

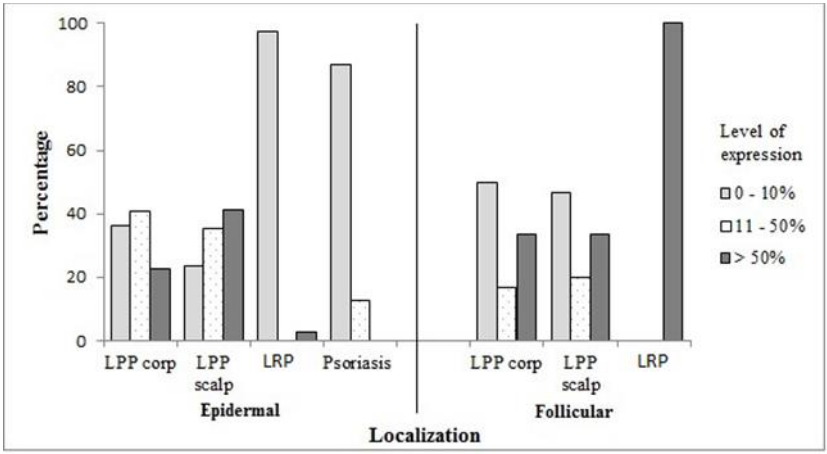

Figure 3.10. **Distribution of CK15 expression levels when epidermal and follicular localization is distinguished in study groups**

Comparing the levels of CK15 expression in two subtypes of LPP and LP, when localization, namely, epidermal or follicular, was not taken into consideration (Fig. 3.11.), we found that LP and psoriasis mostly (92.3 and 87.2 %, respectively) presented with weak CK15 immunopositivity, whereas both LPP types revealed almost equal splitting into the levels of weak, moderate and strong immunopositivity ($\chi 2 = 32.514; df = 4; p < 0.001$).

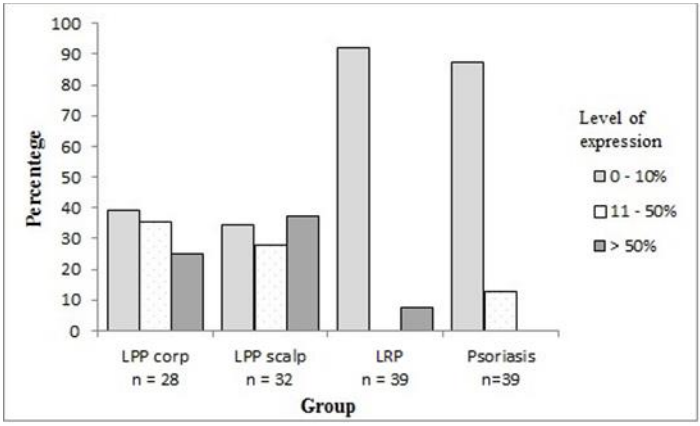

Figure 3.11. **Distribution of CK15 expression in study**

Both LP forms displayed follicular CK15 immunopositivity in the hair follicle bulge region, specifically, the outermost layer of the outer root sheath, which usually was strong, and strong to moderate in the inner root sheath. Moreover, CK15 positive follicular cells revealed a remarkable diminishment when lymphocytic infiltration happened to be localized in the close vicinity. Epidermal expression of CK15 was almost nil in the case of LP, whereas its positive decoration of the cytoplasm of some particular keratinocytes of the basal epidermal layer was demonstrated in LPP. Psoriatic skin samples demonstrated some discontinuous expression of CK15 in keratinocytes constituting the basal layer of epidermis mostly estimated as weak.

3.6. Immunohistochemical analysis of metalloproteinase-9 expression

In all study groups, MMP-9 immunopositivity was distinguished by its brown cytoplasmic colouration, with varying intensity in the epidermis (see Fig. 3.12.).

MMP-9 expression was also observed in other skin structures, varying from negative to intensive. Inflammatory infiltrates displayed intensive, sweat glands – moderate to intensive, sebaceous glands – negative, hair follicles – moderate to intensive and vascular wall – negative to weak expression, respectively.

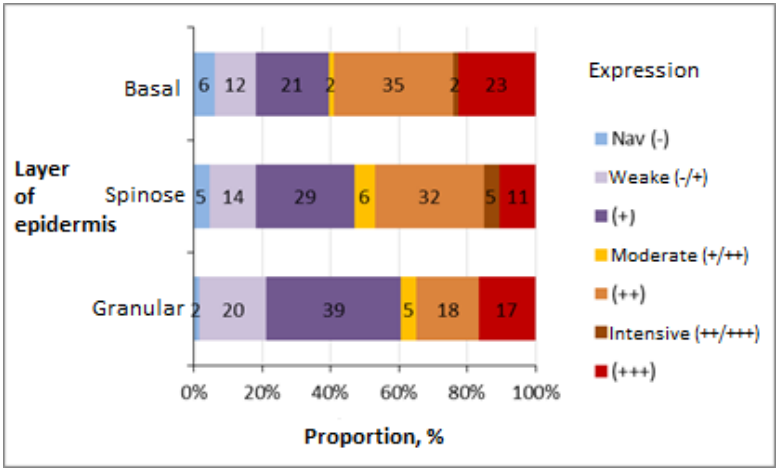

Figure 3.12. **Levels of MMP-9 expression (displayed as the percentage) found in epidermal layers**

Comparison of the expression of MMP-9 in 3 layers of epidermis – basal, spinous and granular – revealed different expression intensities of MMP-9 (see Fig. 3.13.). A statistically significant difference was observed for

the immunohistochemically determined intensity of MMP-9 in LP patients in the epidermis in the granular layer compared to the negative control group ($p < 0.01$). In the majority of cases, the LRP group experienced moderate to intensive (++ / +++) expression of MMP-9 in the granular layer, in the hypertrophic LP group (+ / ++), while in the negative control group – intensive expression (+++). Comparing the expression levels of MMP-9 in the spinous layer, it was concluded that hypertrophic LP cases showed weaker (+) MMP-9 expression than negative control (++) ($p < 0.01$). It should be noted that the spinous expression of MMP-9 in LRP is more pronounced when compared to hypertrophic LP, (++) and (+ / ++) respectively. Comparing the expression of gelatinase between the layers of the epidermis within a single group, a statistically significant difference was observed in the negative control group: basal layer displayed a weaker expression than granular layer ($p < 0.05$, (+ / ++), (+++)), and spinous layer weaker expression than in the granular layer ($p < 0.01$, (++), (+++)), respectively. A different result was observed in the LP group between basal and granular layer – (+ / ++) and (++ / +++) ($p < 0.05$), respectively. The results of immunohistochemical studies showed that the expression of MMP-9 is more intense in the basal layer (++) in relation to the spinous layer (+ / ++).

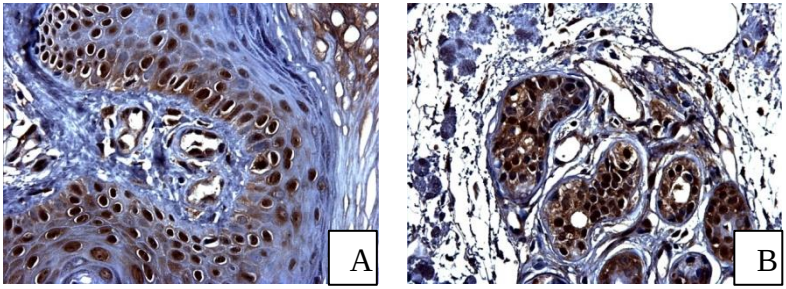

Figure 3.13. **A) LP microphotography: expression of MMP-9**
Increases in direction from suprabasal layers of epidermis (+) to the basal layer (++/+++), × 400
B) LP microphotography: MMP-9 expression in sweat glands, × 400

3.7. Results of the TUNEL reaction and programmed cell death evaluation

TUNEL reaction positivity was distinguished as brownish nuclear staining. Study groups revealed greatly varying TUNEL reaction results summarized in the figure 3.14.

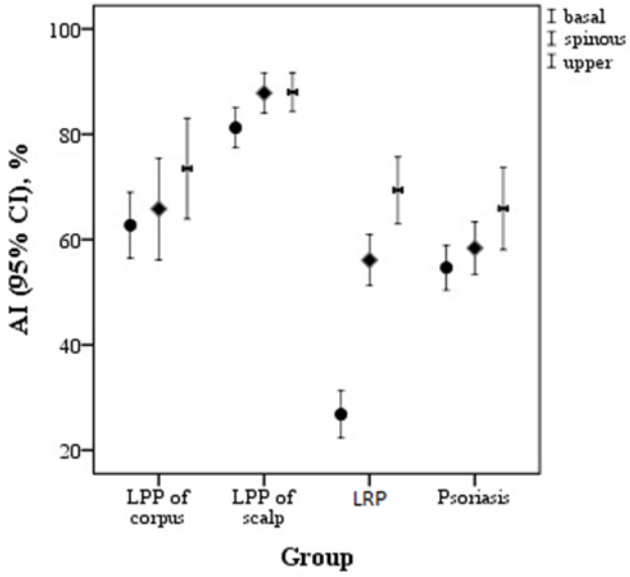

Figure 3.14. **Assessment of AI in the LP, LPP of the scalp, corpus region and psoriasis**

The AI value was lower in the LP group than in the LPP group, revealing an increase in index values from the basal toward the upper epidermal layer (see Fig. 3.15.). The highest estimates were demonstrated for the scalp region of LPP; these were expressed as follows – 81.2 ±10.7; 87.8 ±10.7 and 88.0 ±10.5 for the basal, spinous and upper epithelial layers, respectively. AI dispersion analysis (ANOVA) showed statistically significant differences between patient groups within the basal ($F = 108.7$; $p < 0.001$), spinous ($F = 29.6$; $p < 0.001$), and upper ($F = 10.7$; $p < 0.001$) epidermal layers. In the basal epidermal layer the mean difference was 18.49 (95% CI: 10.60–26.39), 35.91 (95 % CI: 27.36–44.46), 54.41 (95 % CI: 46.62–62.20), 26.57 (95 % CI: 17.56–35.57) and 27.84 57 (95 % CI: 18.19–37.48) when LPP of corpus and scalp, LPP of corpus and LP, LPP of scalp and LP, LPP of scalp and psoriasis, and LP and psoriasis were compared, respectively, whereas in the

spinous layer and upper epithelial layers these variables were observed as 21.67 (95 % CI: 12.05–31.29), 8.78 (95 % CI: from –1.37 to 18.93) and 30.45 (95 % CI: 21.05–39.86), and 13.02 (95 % CI: 2.91–23.13), 5.42 (95 % CI: from –5.24 to 16.09), 18.44 (95% CI: 8.56–28.32), 29.44 (95% CI: 18.21–40.67) and 22.11 (95 % CI: 9.7–34.47), respectively. It should be noted that without the damaged apoptotic keratinocytes, positive TUNEL reaction was observed in intraepithelial lymphocytes and inflammatory infiltrates of the dermis, which were not listed separately in this study.

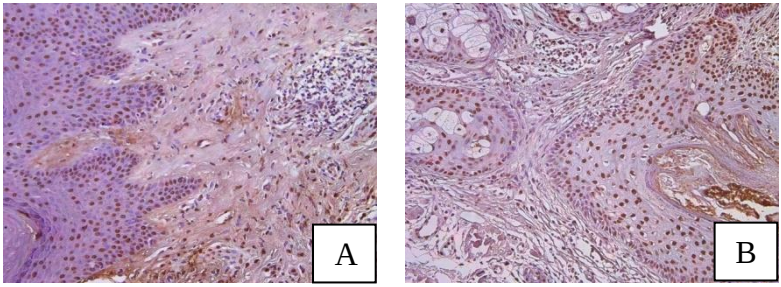

Figure 3.15. **A) LP microphotography: TUNEL+ cells in epidermis and dermal inflammatory infiltrate in case of LRP, × 200**

B) LP microphotography: TUNEL+ cells in case of LPP, × 200

3.8. Epidermal and dermal ultrastructural changes in case of *lichen planus*

Electron microscopy was used to obtain better understanding of the pathogenesis of LP by investigating cell changes at the organoid level and changes in cell-to-cell or cell-to-matrix contacts determining a role of keratinocyte cytoskeleton. Eight patients with LP underwent an ultrastructural analysis based on findings of immunohistochemistry analysis of their biopsy material. Keratinocytes of the basal layer were often separated from the

basement membrane and showed various degenerative changes. The tonofibrillar system appearing in the affected keratinocytes demonstrated a wide spectrum of changes – from thick and densely packed bundles of intermediate filaments attached locally to the desmosomal plaque and the occasional widening of the intercellular spaces up to chaotically distributed and thinned bundles of intermediate filaments, and even free desmosomal complexes (see Fig. 3.16.) in the expanded intercellular spaces separating damaged keratinocytes with low cytoplasmic electron density.

Rounded, oval and irregularly shaped accumulations of tonofilaments with sparse cytoplasmic organelles considered to be CB, were demonstrated in or between the degenerating keratinocytes separated from the underlying basement membrane or in the upper dermis.

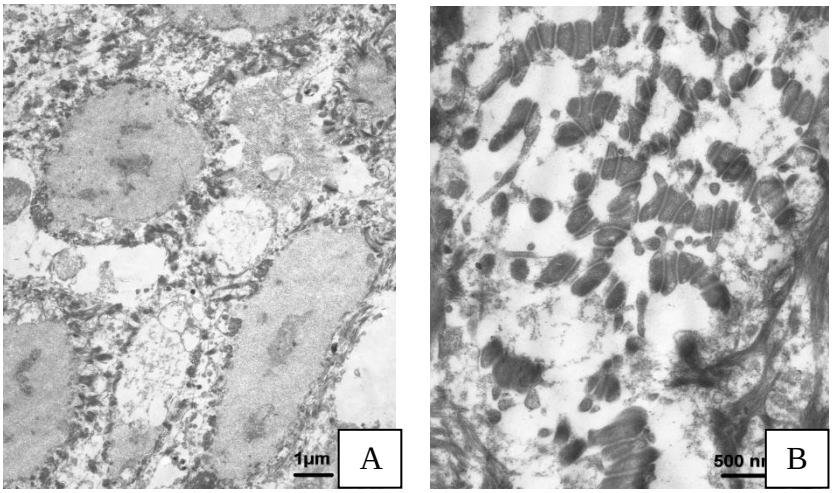

Figure 3.16. **A) LP electronogram: severe damage of the keratinocytes' tonofibrillar system, × 5000; B) free desmosomal complexes in the expanded intercellular space, × 12000**

The altered basement membrane demonstrated a discontinuity with local ruptures or, conversely, multiplications. Occasionally, remnants of the basement membrane were demonstrated freely in the dermis, being surrounded by collagen fiber microfibrils (see Fig. 3.17.). By contrast, in the upper part of the epidermis the intercellular junctions were preserved.

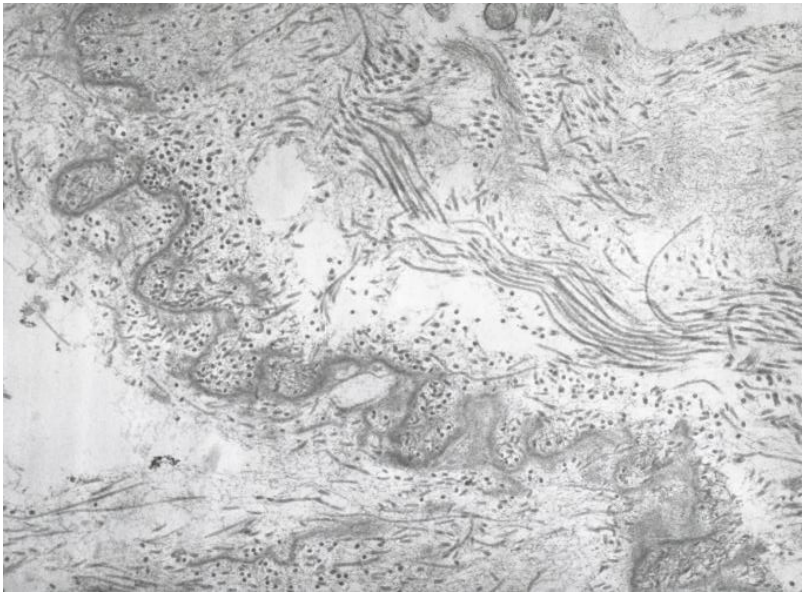

Figure 3.17. **LP electronogram**

Remnants of the basement membrane in dermis, surrounded by collagen fiber microfibrils, × 10000

4. DISCUSSION

The exact incidence of LP is unknown. Nonetheless, it is estimated that LP affects around 0.22 % to 5 % of the world's population. LP is more common in middle-aged people of both sexes, and therefore our study included patients from 16 to 89 years of age. LP was more commonly observed in patients aged 31–60, coinciding with the study by *Arora et al.* (*Arora et al., 2014*). The disease is not pronounced for a particular gender, although some studies indicate that females are more likely to have LP 2:1 (*Shiohara and Kano*, 2008; *Kanwar and De*, 2010). In this study, the dominance of females was also observed. The presence of LP in one family may indicate genetic predisposition (*Bermejo-Fenoll and López-Jornet*, 2006). A positive family history was observed in no patients of this study. LRP was the most common clinically morphological variant of LP, and these data coincide with the results of studies by other authors (*Arora et al.*, 2014). The most commonly skin rash was localized on skin of legs and arms. In the case of any localization of LP, a physician should investigate all potentially included localizations: mucous membrane, skin and skin derivatives (nails and hair). In the case of odynophagia or dysphagia, specialized otorhinolaryngologic and endoscopic examinations should also be considered (*Le Cleach and Chosidow*, 2012). The incidence of LP subtypes varies according to the age and race of the patients. Previous studies have shown that nail and linear LP are more common in children (*Tosti et al*., 2001; *Kabbash et al*., 2002), while pigmented LP – in Latin Americans and other dark-skinned populations (*Pock et al., 2001*). These data supported the distribution and grouping of patients in this study. Only adults were included in the study and patients were White.

A hallmark of LP is an itch, which is usually extremely pronounced or even painful (*Reich et al., 2011*). LP is itchy dermatosis, but some patients do

not have this symptom (*Pock et al., 2001*) or it is not intense (*Chen et al., 2015*). Itch is caused by pruritus triggers that activate nerve fibres (*Sun et al., 2009*). Leukocytes, keratinocytes, mast cells, fibroblasts, endothelial cells, and nerve fibres of the skin can produce several itch stimuls, secreting histamine, quinines, proteases and cytokines (*Ikoma et al., 2006*). Changes in keratinocytes are significant and were observed in all patients of the study, suggesting the importance of these changes in the development of LP itch. In addition, hypergranulosis plays an important role in the development of itch (*Lee et al., 2010*), which also characterizes the morphology of this study material, especially in hypertrophic LP. Referring to world literature (*Kadowaki et al., 2001*), it seems also useful to evaluate the role of DC and lymphocytes in the pathogenesis of itch. As a result of the doctoral thesis, significant presence of DC in the epidermis and the dermis was observed. Inflammation infiltrate dominated morphologically in acute rashes, which may also predispose to itch at the active stage of the disease. Decrease of the disease activity and inflammation should led to reduction of itch. Subjective sensations of the patient, i.e., itching, could indicate the activity of the disease. Summarizing information on skin itch, it should be noted that this is an important LP symptom (*Welz-Kubiak and Reich, 2013*), which has not been studied extensively enough.

Dermatoscopy allows visualization of WS, which is considered as a sensitive and specific criterion for LP diagnosis. WS is observed as round, linear, square or ring-shaped white structures, from which both fine and wide-angle projections can be formed, surrounded by dotted or linear blood vessels that mark the projections out (*Lallas et al., 2014*). In addition, we observed that WS is visible both in the active inflammatory process and in the regressive phase. LP postinflammation hyperpigmentation is not uncommon in patients with III-IV skin phototypes after Fitzpatrick classification. We determined that LP patients often have postinflammation hyperpigmentation. In our opinion,

this sign could be influenced not only by the patient's skin phototype but also by the pathogenesis of LP, the duration and the course of the disease. Dermatoscopic vascular morphology is characterized by dotted and linear blood vessels that interchange and are often placed peripherally in relation to WS. Dermatoscopy is definitely defined as an integral part of examination of LP patients, not only in the diagnosis but also in the course of the disease and, possibly, in the prognosis of the outcome. Acanthosis and fibrosis is an appropriate histopathological sign of WS, but dermatoscopically pink dotted and linear structures correspond to blood vessels with an enlarged cavity. It should be concluded that, when seeing both of these signs in the LP element, one should think of chronic and acute characteristics that exist in the process together in one place. Perhaps WS is a prognostic sign, indicating a more complex process and a longer course of the disease. However, the background colour of the lesion indicates the activity of the disease as well: red – the progression stage, but yellowish – the regression stage.

The distribution of the process, namely, localized or disseminated, is not a commonly used characteristic in the LP description. However, in 1973, *Pinkus* (*Pinkus, 1973*) proposed the term "regional lichenoid syndrome", which also includes LP. In our study, prevalence of the disseminated disease was less common than the localized: 25 (21.4 %) and 92 (78.6 %), respectively, and incidence of the disseminated disease was observed only in LRP patients. This could indicate that vesiculobullous, pigmented, hypertrophic, atrophic LP and LPP are regional diseases. In world's literature (*Garcia-Garcia et al*., 2012), depending on the clinical and morphological signs of the mucous membrane LP, three phases of disease are identified: Phase 1, the initial Phase, with lesions that lasts 6-12 months or more and white dot-like structure in the mucosa; it is followed by Phase 2 – rash duration 10-30 years, clinically WS and histologically lymphocytic inflammation without significant changes in epithelium. This period can be affected by various phases of activity, most

often manifesting with erosions or erythematous mucosal rash. Finally, the late Phase 3 of the disease, which lasts for several years and decades characterized by WS, mucosal atrophy and hyperkeratosis. In our opinion, it is possible to create a similar division of the course in the case of cutaneous LP, analysing in complex the history data, the clinical picture, the results of dermatoscopy and histochemistry. Taking into consideration the results of the doctoral thesis, we have observed the parameters that might be subdivided into the following phases of the disease: progression Phase of the disease 1) clinically cyanotically pink papules; dermatoscopically red skin background, WS, granular pigmentation, linear blood vessels prevalence; morphologically intensive band-like lymphocytic infiltrate and the regression Phase of the disease 2) clinically cyanotic papules or pigmented spots; dermatoscopically light red or yellowish skin background with granular and diffused superficial pigmentation, WS and prevalence of dotted blood vessels; morphologically moderate infiltrate with melanophages. However, more extensive group studies are needed to improve the division of the disease activity and predict the course of the disease. Recent studies advised the use of data of this nature to select a treatment option (*Zouboulis et al.* 2015; *Newlands et al.*, 2016).

Study of the literature on the development of scarring alopecia have led to the understanding that the clones of C8/144B and LHK15 anti-CK15 antibodies are most commonly used in studies (*Misago et al*., 2014), and the clone of the LHK15 antibody, which is recognized as a marker of epithelial stem cell reservoir for humans (*Al-Refu et al.*, 2009) was selected for this study. Determining the presence of fibrosis, which is a common histopathological sign in the case of primary scarring alopecia, it was observed that the inadequacy of CK15 expressivon appear with severe fibrosis. This statement is consistent with previously obtained results (*Hoang et al*., 2009). In this study, negativity of CK15 was shown in inflamated follicles, suggesting that loss of eHFSCs occurs due to inflammation, as similarly it was determined in the study of

Pozdnyakova and Mahalingam (2008) (*Pozdnyakova and Mahalingam*, 2008). Lymphocytes intensively colonize the skin as an organ, but the question is whether and how immune cells affect epithelial stem cells of the skin. Previously published data on eHFSCs controlled by macrophages in the skin, as well as evidence that apoptotic death of macrophages in the early telogen phase leads to stem cell activation, give new information on this topic (*Castellana et al*., 2014). Based on the speculative view, the assumption of the involvement of CK15 positive stem cells in inflammation or, possibly, in the autoimmune mechanism may lead to a new theory on pathogenesis of LPP.

Anti-S100 antibody recognizes epidermal melanocytes and LC, as well as dermal histiocytes. Other authors studied the DC subgroups forming LC (Langerin$^+$, CD1a$^+$), located in the epidermis, together with interstitial DC, more precisely, local dermal myeloid DC (CD11c$^+$, CD1c$^+$), plasmacytoid blood DC (BDCA-2$^+$, CD123$^+$), and dermal populations DC – CD14$^+$ CD11c$^+$ DC found in normal skin, and suggested that skin diseases are characterized by a specific DC profile (*Johnson-Huang et al*., 2009; *Steinman*, 2012). This study analysed the results of immunohistochemical S100 determination in LP cases, studied intraepithelial LC and DC of the dermis, and our data correlate with the results obtained by Santoro with coauthors (2005) (*Santoro et al*., 2005). However, no significant differences between the groups were found in this study and this could indicate a similarity between forms of LP. It was noted that the higher number of S100 positive cells in the study was observed in skin samples with severe infiltration. The low number of S100 positive epidermal cells was found in the psoriatic skin, possibly indicating remission of the disease. The exact biological role of S100 protein in LP is not known. However, the study revealed the immunoexpression of S100 protein in various LP subtypes in the epidermis and in the dermis.

MMP is likely to play a significant role in the production of BM damage in the skin epithelium. T-lymphocytes produce several extracellular matrix-

degrading enzymes with multiple specific substrates (*Gunduz et al*., 2006). The loss of basal keratinocytes and the loss of cellular contacts is a typical morphofunctional indicator of LP, which, in our opinion, correlates with increased immune reactivity of MMP-9 in the skin. *Zhou et al.* (2001) reports that T cells from oral LP lesions secrete more MMP-9 than healthy control and suggested that it could play an important role in the pathogenesis of LP, leading to BM breaks and stimulating apoptosis (*Zhou et al*., 2001). Our results correspond to the data of the *Gunduz* study (*Gunduz et al*., 2006). The reduction of MMP-9 expression in the case of vesiculobullous LP may be because of severe keratinocyte damage due to the formation of bulla in malpighian layer, however, the inflammatory infiltrate shows a high presence of immune markers. Diffuse epidermal expression of MMP-9 in skin samples of healthy individuals has been observed (*Gunduz et al*., 2006), which differ from the data of this study, where more intensive expression was observed in the granular layer of the epidermis compared to the lower layers of the epidermis.

Literature data on the TUNEL assessment analysis suggests the idea that higher number of apoptotic keratinocytes was observed in LP cases comparing to the healthy control group, while lower number was found when LP was compared to other inflammatory diseases (psoriasis and skin *lupus erythematosus*) (*Kawashimaa et al*., 2004; *Gündüz et al.*, 2006; *Toberer et al*., 2013). Performing TUNEL reaction and AI calculations, an increased number of apoptotic keratinocytes was observed in LPP cases compared to the LRP. The obtained results of TUNEL reaction are consistent with the evidence obtained by *Bloor et al.* (1999) and confirmed by *Harries et al.* (2013), pointing to the increased TUNEL positive epithelial cell counts in cases of LP (*Bloor et al*., 1999; *Harriot and Paus*, 2010). Results of TUNEL reaction, which were confirmed by the AI determination and ultrastructural analysis suggest that, among the groups of patients analysed within this study,

basal and suprabasal keratinocyte damage was particularly pronounced in LPP cases thus explaining with high probability the loss of hair. Without denying the fact that the death of keratinocytes is caused by the expressed cytotoxic factors, electron microscopy investigations were performed to achieve the aim of the study, emphasizing the ongoing role of non specific responses, manifesting in keratinocytes supporting basement membrane damage, changes of cellular cytoskeleton and loss of intercellular contacts.

PRACTICAL RECOMMENDATIONS

1. Patients with cutaneous LP were recommended to collect information about skin itch to understand the activity level of the disease and inflammation. Severe itch of the skin indicates a progression of the disease and serious inflammation of tissue, whereas decreases of itch is associated with lower level of disease activity.
2. Patients with clinical LP were recommended to perform skin biopsy and histochemical analysis of the skin to determine the clinically morphological subtype which is essential in determination of progress of the disease.
3. Patients with follicular LP in addition to routine histochemical analysis were recommended to perform dyeing of tissue with toluidine blue for better evaluation of placement and amout of elastic and collagenic fibre in order to consider about tissue fibrosis.
4. Patients with LP were recommended to use dermatoscopy in diagnostical purposes to determine diagnostical parameters: background color of the skin, Wickham striae, morphology and placement of blood vessels, hyperpigmentation of the skin.
5. Patients with cutaneous LP were recommended to use dermatoscopy in prognostic purposes in determining types of hyperpigmentation in the skin. Dermatoscopically it is possible to distinguish two different hyperpigmentation types in LP: brownish superficial form which is localized in epidermis and granule type which corresponds to dermis melanophages and which are more stable in comparison to the superficial hyperpigmentation after inflammation.
6. Patients with cutaneous LP were recommended to determine the activity level of disease basing on the acquired clinical, dermatoscopical and histochemical results: phase of progression – with clinically cyanotically pink papules; dermatoscopically pink background of skin, Wickham striae,

in some places granule type pigmentation, linear blood vessels; morphologically intense band-like lymphocyte infiltration and phase of regression of the disease – with clinically cyanotical papules or pigmented spots; dermatoscopically light red or yellowish background of skin with granule type and dispersed pigmentation, *Wickham striae* and dominating dotted blood vessels; morphologically with moderate infiltration and commonly with melanophages.

CONCLUSIONS

1. Patients with cutaneous *lichen planus* most commonly are females over 31 years of age with localized lichen ruber planus subtype; clinically, patients demonstrate polygonal, cyanotically pink, shiny papules most commonly on skin of limbs. The most common complaint is pruritus, while regression of *lichen planus* is characterized by hyperpigmented brown spots.
2. Dermatoscopically, inflammatory rashes characteristic of cutaneous *lichen planus* appear as Wickham striae on red or light-red background, peripherally placed dotted and linear blood vessels and superficial dispersed pigmentation.
3. Cutaneous *lichen planus* is most often characterized by hyperkeratosis, compact orthokeratosis, hypergranulosis, irregular acanthosis, basal cell vacuolization, keratinocytes' apoptosis, subepidermal band-like lymphoplasmocytic inflammation and occasional eosinophilic leukocytes.
4. In patients with cutaneous *lichen planus,* CK15 expression decreases in the proximity of inflammatory infiltrate, indicating the association of immune system cells with the keratinocyte differentiation and their role in the pathogenesis of the disease; the CK15 marker is an essential complementary diagnostic and prognostic indicator in cases of alopecia.
5. Tissue material of patients with cutaneous *lichen planus* reveals stronger and denser S100 immunoreactivity comparing to psoriasis or healthy controls. Furthermore, the lesions from the scalp region of LPP patients demonstrated more pronounced immunoreactivity when compared to the corpus, indicating the involvement of immune cells in the pathogenesis of *lichen planus* and even the possible autoimmune nature of the disease.
6. Increased expression of MMP-9 in the basal layer of the epidermis was demonstrated in cutaneous *lichen planus,* thus stressing a role of this enzyme in the destruction of basal keratinocytes and deepening our knowledge on pathogenesis of *lichen planus*.

7. Estimation of apoptotic cells in patients with cutaneous *lichen planus* demonstrates differences in keratinocyte death found in different *lichen planus* subtypes. The highest apoptotic index was estimated in the LPP group, and especially in the scalp region of LPP patients, showing an increase of the estimates from basal to upper epidermal layers and allowing partly explain the development of alopecia in these patients. The electron microscopic investigation of the material confirmed the ultrastructural results of earlier studies regarding the presence of colloidal bodies and innovatively described degenerative changes in the basal layer keratinocytes and the basement membrane in patients with cutaneous *lichen planus*, accentuating peculiarities in differentiation of keratinocytes and the role of cytoskeleton-dependent injury manifested as loss of intercellular contacts in pathogenesis of LP.
8. For patients with clinically defined LP, a skin biopsy followed by tissue analysis is recommended correlating these findings with dermatoscopy results in order to better judge the course and prognosis of the disease, especially in case of development of fibrosis and scarring alopecia.

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PUBLICATIONS AND PRESENTATIONS

Published articles (3)

1. **Leguša, I.**, Groma, V., Mikažāns, I., Hartmane, I. 2012. Matrices metaloproteināzes 9 (MMP-9) ekspresija dažādos ādas lichen planus klīniskajos variantos. *Rīgas Stradiņa universitātes Zinātniskie raksti*. 1, 72–79.
2. **Leguša, I.**, Groma, V. 2012. Matrix metalloproteinase 9 (MMP-9) is differently expressed in cutaneous lichen planus and lichen sclerosus. *Papers on Anthropology* XXI. 176–186.
3. **Upeniece, I.**, Groma, V., Skuja, S., Cauce, V. 2016. Eradication of damaged keratinocytes in cutaneous lichen planus forms demonstrated by evaluation of epidermal and follicular expression of CK15, indices of apoptosis and regulatory protein S100. *Polish Journal of Pathology*.67(3), 258–269.

Abstracts and participation in international congresses and conferences (15)

1. Groma, V., **Leguša, I.**, Mikažāns, I. 2012. Ultrastructual findings in various types of cutaneous lichen planus and their correlation with expression of matrix metalloproteinase (MMP-9). *Conference on Diagnostic Electron Microscopy Basic Research & Oncology - Ultrapath XVI*. Tēžu grāmata. (Stenda referāts).
2. Groma, V., **Leguša, I.**, Mikažāns, I. 2012. Ultrastructure of cutaneous lichen planus lesions evidenced in various types of disease correlated with

tissue levels of gelatinase-B. *24th European Congress of Pathology*. Tēžu grāmata. (Stenda referāts).

3. **Legusa, I.**, Groma, V., Mikazans, I., Semjonova, D., Cerina, E. 2013. Cutaneous lichen planus lesions: clinical and morphological variations and occurrence of matrix metalloproteinase-9 expression. *10th European Academy of Dermatology and Venereology (EADV) Spring Symposium*. P-329, Tēzu grāmata. (Stenda referāts).
4. **Legusa, I.**, Atsledzina, L., Groma, V., Mikazans, I., Hartmane, I. 2013. Pigmented features of lichen ruber planus in dynamics: a pilot study of twelve patients. *22st European Academy of Dermatology and Venereology (EADV) congress*. P-802, Tēžu grāmata. (Stenda referāts).
5. **Leguša, I.**, Mikažāns, I. 2013. Diagnosis and prognosis of cutaneous lichen planus. *2nd International medical meeting*. P-20, Tēžu grāmata. (Mutisks ziņojums).
6. **Legusa, I.**, Groma, V. 2013. Pigmentation in lichen ruber planus: dermoscopic and microscopic interplay. *40th anular meeting of SCUR, 6th Joint meeting*. P-07, Tēžu grāmata. (Stenda referāts).
7. Groma, V., **Legusa, I.**, Ivanova, A., Nora-Krukle, Z., Murovska, M. 2013. Associatin of lichen planus with human herpesvirus type 6 – new implications for therapy. *40th anular meeting of SCUR, 6th Joint meeting*. P-20, Tēžu grāmata. (Stenda referāts).
8. Groma, V**., Legusa, I.**, Tarasovs, M. 2013. Ultrastructural contributions to the characterization of pathogenesis of lichen planus: classics and modern data. *40th anular meeting of SCUR, 6th Joint meeting*. O-12, Tēžu grāmata. (Mutisks ziņojums).
9. **Legusa, I.**, Skuja, S., Groma, V. 2013. MMP-9 in lichenoid cutaneous disorders: overlap and deviation of expression. *25th European Congress of Pathology*. P-275, Tēžu grāmata. (Mutisks ziņojums).

10. **Upeniece, I.**, Mikazans, I., Groma, V., Hartmane, I. 2013. Lichen ruber planus histopathology. *11th Congress of the Baltic Association of Dermatovenereologists (BADV)*. P- 76, Tēžu grāmata. (Mutisks ziņojums).
11. **Upeniece, I.**, Groma, V., Skuja, S. 2013. Lichen planopilaris pathomorphology, MMP-9 expression ans its relevance in understanding of pathogenesis. *Baltic Morphology VII Scientific Conference*. P- 62, Tēžu grāmata. (Mutisks ziņojums).
12. **Upeniece, I.**, Groma, V., Skuja, S., Bondare, L., Mikazans, I., Hartmane, I. 2014. New implications in understanding the pathogenesis of Lichen planopilaris. *12th Congress of the Baltic Association of Dermatovenereologists (BADV)*. P-58, Tēžu grāmata. (Mutisks ziņojums).
13. **Upeniece, I.**, Groma, V., Mikazans, I., Cauce, V. 2015. Lichen planus affecting Latvian adults: clinical, dermoscopical and histopathological data. *5th Euro-Asian assiciation of Dermatovenerologists congress*. P-54, Tēžu grāmata. (Mutisks ziņojums).
14. **Upeniece, I.**, Groma, V. 2015. Cutaneous lichen planus: histopathological observations. *8th Baltic Morphology Scientific Conference*. P-130. Tēžu grāmata. (Stenda referāts).
15. **Upeniece, I.**, Groma, V. 2016. Cutaneous lichen planus: histopathologicalnand ultrastructural observations. *25th European Academy of Dermatology and Venereology (EADV) Congress*. P-0970, Tēžu grāmata. (Stenda referāts).

Abstracts and participation in local congresses and conferences (7)

1. **Leguša, I**., Mikažāns, I., Groma, V. 2012. Lichen planus dermaskopiskās diagnostikas un slimības gaitas prognozēšanas iespējas. *RSU zinātniskā konference*. P-57, Tēžu grāmata. (Stenda referāts).
2. **Leguša, I**., Groma, V., Mikažāns, I. 2013. Lichen planopilaris Latvijā: 15 gadījumu klīniskā un morfoloģiskā analīze. *RSU zinātniskā konference*. P-58, Tēžu grāmata. (Stenda referāts).
3. **Upeniece, I**., Groma, V., Mikažāns, I., Skuja, S. 2014. Programmēta keratinocītu nāve Lichen planopilaris gadījumā. *RSU zinātniskā konference*. P- 53, Tēžu grāmata. (Stenda referāts).
4. **Upeniece, I**., Groma, V., Skuja, S., Cauce, V., Mikažāns, I., Bule, V. 2015. Lichen planus imunoloģiskie aspekti. *RSU zinātniskā konference*. P- 22, Tēžu grāmata. (Stenda referāts).
5. **Upeniece, I**., Mikažāns, I., Cauce, V. 2016. Ādas lichen planus klīniskais rakstirojums. *RSU zinātniskā konference*. P- 23, Tēžu grāmata. (Stenda referāts).
6. **Upeniece, I**., Groma, V. 2016. Epidermas ultrastrukturālas izmaiņas ādas lichen planus gadījumā un atradnes korelācija ar MMP-9 ekspresiju. *RSU zinātniskā konference*. P- 24, Tēžu grāmata. (Stenda referāts).
7. **Upeniece, I**., Mikažāns, I., Groma, V. 2017. Ādas lichen planus klīniski morfoloģiskais raksturojums. *RSU zinātniskā konference*. P- 297, Tēžu grāmata. (Stenda referāts).

APPENDICES

Ethics Committee permission

Veidlapa Nr. E-9 (2)

RSU ĒTIKAS KOMITEJAS LĒMUMS

Rīga, Dzirciema iela 16, LV-1007
Tel. 67409089

	Komitejas sastāvs	Kvalifikācija	Nodarbošanās
1.	Asoc. prof. Olafs Brūvers	Dr.theo.	teologs
2.	Profesore Vija Sīle	Dr.phil.	filozofs
3.	Docente Santa Purviņa	Dr.med.	farmakologs
4.	Asoc. prof. Voldemārs Arnis	Dr.biol.	rehabilitologs
5.	Profesore Regīna Kleina	Dr.med.	patalogs
6.	Asoc. prof. Guntars Pupelis	Dr.med.	ķirurgs
7.	Asoc. prof. Viesturs Liguts	Dr.med.	toksikologs

Pieteikuma iesniedzējs: **Dr. Ilze Leguša,** 2. gada rezidente
Dermatoloģijas, veneroloģijas fakultāte

Pētījuma nosaukums: „Dažādu ādas lichen plānus klīnisko variantu morfoloģiskā un dermaskopiskā izpēte slimības gaitas prognozēšanas un ārstēšanas uzlabošanai".

Iesniegšanas datums: 08.08.2012.

Pētījuma protokols: Izskatot augstāk minētā pētījuma pieteikuma materiālus ir ir redzams, ka pētījuma protokols iesniegts profesionāli augstā kvalitātē. Mērķis tiek sasniegts veicot ar pacientiem (bez kāda apdraudējuma veselībai) klīniski-analītisku darbu, medicīnisko dokumentācijas datu apstrādi un analīzi, kā arī izsakot priekšlikumus. Pacientu (dalībnieku) personas datu aizsardzība, brīvprātīga informēta piekrišana piedalīties pētījumā un konfidencialitāte tiek nodrošināta. Līdz ar to pētījuma pieteikums atbilst medicīnas ētikas prasībām.

Pētījuma populācija: nav norādīta

Komitejas lēmums: **piekrist pētījumam**

Komitejas priekšsēdētājs Olafs Brūvers Tituls: Dr. miss., asoc. prof.

Paraksts

Ētikas komitejas sēdes datums: 27.09.2012.