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Collagen and elastin differences in vulvar tissue of women with lichen planus, lichen sclerosus and healthy women

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ABSTRACT

Objectives: Lichen sclerosus and lichen planus are two debilitating dermatoses. Their etiology remains unknown. Skin changes resulting from these disorders are important to understand, so we can provide targeted treatment to patients.

We examined the differences in collagen (*COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL5A3*) and elastin (*ELN*) expression between vulvar tissue of women with lichen planus, lichen sclerosus and healthy women.

Material and methods: Vulvar tissue was taken from areas affected by lichen planus or lichen sclerosus. In healthy controls, we biopsied vulva at five and eight o'clock in a standardized manner. The tissue was simultaneously sent for pathological and genetic analysis. When either lichen planus or sclerosus or healthy tissue was confirmed by pathologist, we processed the genetic sample. RNA was isolated, transcribed and gene expression was analyzed using Real Time Custom Panel 96-16 and LightCycler 480 Probe Master. Kolmogorov-Smirnov test was employed to determine if the data on the population show normal distribution. For genes with normal distribution, t-Test was employed and for those lacking normality, we used Mann-Whitney 1-tail test. The threshold for p value was set less than 0.05.

Results: Thirty-nine vulvar samples were examined. The mean expression of *COL1A1* was 11.13, *COL1A2* was 6.72, *COL3A1* was 8.43, *COL5A1* was 11.91, *COL5A2* was 10.62 and *COL5A3* was 12.79. The mean expression of elastin (*ELN*) was 13,13. We found statistically significant difference in expression of collagen (*COL1A2)* and elastin (*ELN)* between healthy controls and patients with lichen planus ($p = 0.4$). We did not find differences for other genes ($p < 0.05$).

Conclusions: Collagen and elastin are differentially expressed between patients with lichen planus and healthy controls.

Keywords: collagen; elastin; lichen sclerosus; lichen planus; vulva

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INTRODUCTION

Collagen, as a main component of the extracellular matrix, remains the most widespread protein in the human body. Its distinguishing feature is the presence of a long triple-helical domain. Twenty-eight types of collagens have been identified. The amount of triple helical component may vary from as little as under 10% of the whole collagen structure in collagen type XII to as much as 96% in collagen type I. Apart from forming supramolecular assemblies in the extracellular matrix, some collagens exist in a soluble form [1].

Elastin is another major element of the extracellular matrix. Contrary to collagen, elastin provides tissues with elasticity. Elastin is produced mainly by fibroblasts in the form of its precursor called tropoelastin. To create a complete elastin molecule, tropoelastin molecules must undergo aggregation and crosslinking processes. Elastin is a crucial protein in ligaments, tendons, blood vessels and lung tissue. Similarly, to collagen, elastin mutations lead to a variety of disorders affecting different parts of the body [2].

Lichen planus (LP) is a T-cell mediated inflammatory dermatosis that affects both keratinized and nonkeratinized

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squamous epithelium. Three types are described on the vulva: erosive, classic, and hypertrophic [3]. Lichen sclerosus (LS), similarly to LP is a T-cell–mediated inflammatory dermatosis, however it is directed against unknown epitopes on basal cells of squamous epithelium. Typically, LS affects anogenital skin of women and girls but may also occur on extragenital sites and in males [4].

Previously, damage of collagen IV has been described in oral lichen planus due to an inflammatory response [5]. This leads to destruction of the epithelial-connective tissue interface in oral LP. Furthermore, antibodies against collagen XVII are present in lichen planus [6].

Autoantibodies against the extracellular matrix protein 1 (ECM 1) and basement membrane antigens BP180 and BP230 have been shown to play a role in pathophysiology of lichen sclerosus. ECM1 autoantibodies lead to disruption of the basement membrane by affecting the binding of ECM1 to proteins (*i.e.*, collagen IV) at the dermoepidermal junction (DEJ). Another pathogenetic mechanism in LS is stimulation of fibroblasts resulting in abnormal collagen synthesis and increased hyalinization and sclerosis in the skin [7].

In lichen planus, the characteristic feature is a scarcity of elastin fibers, varying from a partial reduction to complete destruction in the papillary dermis [8]. Vulvar tissue affected by lichen sclerosus showed reduced numbers of elastin fibres [9].

Collagens and elastins in the skin are probably heavily affected by vulvar LS yet is less investigated in LP. It seems important to investigate the gene expression of collagens and elastin in women with LP and LS to compare if they are differently expressed. Thus, our aim was to compare the expression of the following genes: collagen 1A1 (*COL1A1*), collagen 1A2 (*COL1A2*), collagen 3A1 (*COL3A1*), collagen 5A1 (*COL5A1*), collagen 5A2 (*COL5A2*), collagen 5A3 (*COL5A3*), elastin (*ELN*) and *ECM1* in vulvar tissue of women with lichen planus, lichen sclerosus and in healthy vulvar tissue.

MATERIAL AND METHODS

The study was conducted between 2018 and 2021 among women who were admitted to the Department of Gynecology, Obstetrics and Oncological Gynecology, Medical University of Silesia, Bytom, Poland for invasive diagnostic procedures related to their complaint of vulvar pruritus or irritation. After pathologic confirmation of lichen planus or lichen sclerosus, vulvar tissue was further processed as described below. The control group consisted of women who had gynecological procedures performed due to other indications and agreed to provide vulvar biopsy for the purpose of this study. After the pathologic confirmation that the vulvar tissue was normal, it was processed as described below. Exclusion criteria included: lack of consent, pathologic report indicating disorders other than lichen sclerosus or lichen planus or defining any type of pathologic abnormality in case of controls.

The vulvar biopsies were taken from areas suspected of disease or in case of controls from macroscopically healthy vulva at five and eight o'clock by an experienced gynecologist under local anesthesia with one percent lidocaine. Vulvar biopsies were immediately divided into two probes for standard pathological analysis to confirm the diagnosis and for genetical analysis.

The study was approved by Ethical Committee of Medical University of Silesia, Katowice, Poland.

Genetic analysis

The genetic assessment procedure was described previously by our group with details [10].

The following genes were examined: *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, *COL5A2*, *COL5A3*, *ELN* and *ECM1*.

Statistical analyses

To confirm the normal distribution within the examined genes we applied Kolmogorov-Smirnov test. In genes with normal distribution, we t-Test was employed. For the data not normally distributed the non-parametric test: Mann-Whitney 1-tail test was engaged. The threshold for p value was set less than 0.05.

RESULTS

Total of 39 vulvar samples were assessed. Table 1 presents collagen gene expression.

The mean level of *ELN* was 13.13 (SD $\pm$ 2.16) and *ECM1* was 10.24 (SD $\pm$ 1.73).

In genes with normal distribution, we used t-Test and found no statistical difference for comparison between control and lichen planus samples, control and lichen sclerosus samples and between LP and LS samples for *COL1A1*, *COL5A1*, *ECM1* ($p > 0.05$). Data shown in Table 2.

In genes without normal distribution, we used Mann Whitney test and found statistically significant difference for *COL1A2* and *ELN* in controls vs LP ($p = 0.4$). Other genes (*COL3A1*, *COL5A2*, *COL5A3*) did not show statistical difference between the groups. Data presented in Table 3.

Fold change was negative for *COL1A2* and *ELN* in comparison between controls and LP (respectively, –3.566, –2.336). The mean expression of *COL1A2* in controls was 5.91 and 7.74 in LP patients. The mean expression of *ELN* in controls was 12.60 and *ELN* 13.83 in LP patients.

DISCUSSION

Both vulvar lichen planus and lichen sclerosus are debilitating diseases with still unknown pathophysiology. Our study adds new information to unveiling skin changes that appear in these disorders. We showed increased expression

Table 1. Analysis of collagen genes normalized to two reference genes: *GAPDH* and *RN18S1*

	COL1A1	*COL1A2*	*COL3A1*	*COL5A1*	*COL5A2*	*COL5A3*
Average	11.13	6.72	8.43	11.91	10.62	12.79
SD	3.08	2.66	2.7	1.96	2.25	1.58
Normality test	Yes	No	No	Yes	No	No

SD — standard deviation

Table 2. Gene expression comparison between lichen planus (LP), lichen sclerosus (LS) and controls in genes with normality distribution

	Controls vs LP	Controls vs LS	LP vs LS
	p value	p value	p value
COL1A1	0.186848788	0.126708	0.442694
COL5A1	0.124498298	0.434763	0.177186
ECM1	0.474049492	0.161499	0.214463

Table 3. Gene expression comparison between lichen planus (LP), lichen sclerosus (LS) and controls in genes without normality distribution

	Controls vs LP	Controls vs LS	LP vs LS
	p value	p value	p value
COL1A2	0.044748	0.277867	0.180029
COL3A1	0.115	0.249369	0.252821
COL5A2	0.075704	0.263422	0.194954
COL5A3	0.441809	0.473902	0.380151
ELN	0.037058	0.388327	0.106002

of *COL1A2* and *ELN* in vulvar lichen planus in compare with healthy vulvar tissue. It is important to focus on the role of *COL1A2* and *ELN*. COL1A2-derived protein promotes fibroblast cell proliferation and collagen type I synthesis [11]. It also enhances wound healing and elastin production. However, it should be noted that *COL1A2* is highly expressed in cancer cells [12] and COL1A2 antibodies is highly present in serum of patients with glioblastoma [13]. Thus, our finding of increased expression of *COL1A2* in patients with LP comparing to healthy tissue may indicate the possible precancerous changes in LP cells. However, this requires further, targeted both *in vitro* and *in vivo* studies. The prevalence of simultaneous LP and vulvar squamous cell cancer ranges from 1 to 33% [14], therefore expanding knowledge about this disease related to its underlying mechanism may be important.

On the other hand, COL1A2 has an important role in fibroblast stimulation and activation. It was proven that lncRNA COL1A2-AS1, also known as lncRNA8975-1, inhibits hypertrophic scar fibroblast proliferation [15]. So, when the expression is downregulated, it may lead to improper healing mechanism, through disruption of fibroblast apoptosis [15]. The role of upregulated *COL1A2* expression in patients with LP remains unknown for fibroblast proliferation and should be further investigated. Our findings should be accounted in assessing the role of COL1A2, aside of TGF-β and other markers on skin fibroblasts [16].

Another finding was that elastin gene expression (*ELN*) is elevated in LP comparing to healthy vulvar tissue. It is interesting as loss of dermal elastin fibers was described both in vulvar LS and LP [17]. Furthermore, elastin is decreased in patients with oral LP comparing to buccal normal mucosa, with increased levels of neutrophil elastase in patients with LP [18]. Thus, we hypothesize that the elevation of elastin gene expression in vulvar tissue of patients with LP may also evoke from increased elastin degradation and secondary rebound.

CONCLUSIONS

Finally, our research adds new information about skin changes in vulvar lichen planus and lichen sclerosus which may prompt further studies to better understand cell changes in these diseases. It may help guide targeted therapies for women suffering from these disorders.

Article information and declarations

Data availability statement

Data available within the article.

Ethics statement

The study was approved by the Ethics Committee at Medical University of Silesia

Author contributions

Marzec Adrianna: data analysis, manuscript writing.
Augusciak-Duma Aleksandra: project development, samples processing, data analysis, manuscript writing.
Lubik Dominika: data collection.
Olejek Anita: project development.
Gabriel Iwona: project development, data analysis, manuscript writing.

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None.

Conflict of interest

Authors report no conflict of interest.

Supplementary material

None.

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