Study of Expression of Suppressor of Cytokine Signaling 3 in Lichen Planus

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Abstract

Background: Lichen planus (LP) is a mucocutaneous disease that is relatively common between adult populations. LP can present as skin and oral lesions. **Objective:** The present study aimed to detect the expression of SOCS3 in lichen planus of diseased patients as compared to normal control persons. **Patients and methods:** This study was a case control study included 30 Lichen planus patients as cases chosen from outpatients attending Dermatology Department at Beni- Suef University Hospital and 30 normal as controls calculated by sample size calculator formula. **Results:** In our study, LP cases and control were compared using SOCS3; to determine the cut-off value, we performed a ROC analysis. SOCS3 has perfect value for discrimination between LP cases and controls with an area under the ROC curve of 1.0 (95% CI = 0.940 to 1.000, P-value <0.0001). Best cut-off is a value of 1.5 (sensitivity = 100%, specificity = 1.0, J-index = 1.0). **Conclusion:** Our results were unique in studying the role of SOCS3 in skin form of LP. SOCS3 has perfect value for discrimination between LP cases and controls.

Keywords: Suppressor of Cytokine Signaling 3, Lichen Planus.

1. Introduction

Lichen planus is characterized by lichenoid, polygonal papules with fine white lines, named Wickham striae. Lesions most commonly occur on the limbs and on the trunk dorsal aspect. At the same time often leukoplakia of mucous membranes as well as nail disorders are present. There are numerous variants of lichen planus which can be distinguished from the classical form on the basis of morphology and distribution of the lesions [1]. One of the main histopathological features of LP is the keratinocytes vacuolar degeneration in the basal layer. This phenomenon is the result of the action of T helper lymphocytes, T cytotoxic lymphocytes, natural killer cells and dendritic cells that predominate in the inflammatory infiltrate. So, the main pathogenic mechanisms

are increased apoptosis of keratinocytes and the inhibition of apoptosis of T lymphocytes [2]. Suppressor of cytokine signaling (SOCS3) is known to inhibit cytokine signaling through Janus kinase (JAK)/signal transducers, activators of transcription [3], NFkB, and focal adhesion kinase (FAK) signaling pathways. Substantial data demonstrated the link between SOCS3 regulation of inflammation and its suppressor activity on tumor initiation and development [4]. Methylation of the SOCS3 promoter region resulted in the decreased expression levels of SOCS3 mRNA in oral Lichen planus [5], suggested that frequent methylation of the SOCS3 gene promoter, theoretically resulting in cytokines expression increase, might be associated with etiological mechanism of oral Lichen planus.

2. Aim of the study

The present study aimed to detect the expression of SOCS3 in lichen planus of diseased patients as compared to normal control persons to investigate a possible role of SOCS3 in the pathogenesis of this disease.

3. Patients and methods

Patients: The present study was conducted after the approval of the medical and Ethical Committee, and patient consent. This study was a case control study included 30 Lichen planus patients as cases chosen from outpatients attending Dermatology Department at Beni-Suef University Hospital and 30 normal as controls calculated by sample size calculator formula which is:

$$\frac{r+1}{r}\frac{SD2(z\beta + Z\alpha/2)2}{d2}$$

r: ratio between cases & controls

SD: standard deviation from previously published study.

Zβ: Standard normal variety for power (0.84).

$Z\alpha/2$: Standard normal variety for level of significance (1.96).

d: absolute error or precision.

Inclusion criteria were:

- Age between 20 to 60 years
- Both males and females
- Patients with lichen planus of skin
- Patients not receiving systemic or topical treatment for lichen in the last three months

Exclusion criteria:

- Age below 20 and above 60 years
- Patients with other types of lichen planus.
- Patients receiving systemic or topical treatment in the last three months.
- Patients with confirmed other autoimmune diseases

Controls were chosen randomly from any other outpatient clinics (who are apparently normal and matching with the age).

Methods:

All the patients and healthy controls were subjected to the following:

- A written consent for participation in study
- Detailed history taking

- Clinical assessment to determine type, extent and sites of lichen planus

Four mm punch skin biopsy with local anesthesia were taken from patients (lichen planus) & control (from site corresponding to the lesion) but area of the face was excluded from both patients and controls and it was kept in lysis solution for the stability of the studied parameters and was kept frozen at -80 Celsius till analysis of SOCS3 by real time –PCR [5].

Quantitative real time PCR:

RNA extraction:

Total RNA was isolated using Qiagen tissue extraction kit (Qiagen, USA) according to instructions of manufacture.

1. Thirty mg of the animal tissue sample was excised and placed directly into a suitably sized vessel for disruption and homogenization**.**

2. The tissue was disrupted, lysed in lysis Buffer RLT and the lysate was homogenized by tissue homogenizer for 40 seconds.

3. The lysate was centrifuged for 3min. at full speed and the supernatant was carefully removed and transferred into a new micro centrifuge tube.

4. 350 µl of 70% ethanol was added to the cleared lysate.

5. 700 µl of the sample was transferred to an RNeasy spin column placed in 2 ml collection tube and centrifuged for 15 sec. at $\geq$ 8000 rpm.

6. 700 µl Buffer RW1 was transferred to the RNeasy spin column and Centrifuged for 15

sec. at $\geq$ 8000 rpm to wash the spin column membrane.

7. 500µl Buffer RPE was added to the RNeasy spin column, and centrifuged for 15 s at $\geq$ 8000 rpm to wash the spin column membrane.

8. 500µl Buffer RPE was added to the RNeasy spin column and centrifuged for 2 min at $\geq$8000 rpm to wash the spin column membrane.

9. RNeasy spin column was placed in a new 1.5 ml collection tube. 30–50µl RNase-free water was added directly to the spin column membrane, and centrifuged for 1 min at $\geq$8000 rpm to elute the RNA.

10. The eluate was transferred to a new Eppendorf tube and stored at –80 °C for further use.

The purity (A260/A280 ratio) and the concentration of RNA were obtained using spectrophotometry (dual wave length Beckman, Spectrophotometer, USA).

cDNA synthesis: The total RNA (0.5–2 µg) was used for cDNA conversion using high capacity cDNA reverse transcription kit Fermentas, USA).

Real-time qPCR using SYBR Green I: Real-time qPCR amplification and analysis were performed using an Applied Bio system with software version 3.1 (StepOne™, USA). The qPCR assay with the primer sets were optimized at the annealing temperature.

Statistical analysis: Recorded data were analyzed using the statistical package for social

sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance was used when comparing between two means.

- Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.

- The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:

- Probability (P-value)

- P-value <0.05 was considered significant.

- P-value <0.001 was considered as highly significant.

- P-value >0.05 was considered insignificant.

4. Results

The current study included 30 Lichen plannus patients. They all presented to dermatology department at Beni-Suef University hospital. Their age ranged from 21 to 64 years. Thirty healthy controls were taken. Detailed history and clinical examination were performed. Skin biopsies were collected and SOCS3 levels were assessed and compared in both groups.

Clinical data of the study group:

Age and sex of LP group: The age of LP cases ranged from 21 to 64 years with mean age of 39 years. Regarding sex, 28 cases (93.33%) were males (mean was 5.68, SD was 2.57) and 2 cases (6.66%) were females (mean was 6.40, SD was 4.38) and P-value 0.714, as shown in Table (1).

Table (1): Relation between SOCS3 and categorical variables in cases of LP

Variable		n	SOCS3 Mean	SD	P-value*
Gender	Male	28	5.68	2.57	0.714
	Female	2	6.40	4.38	
Smoking	Non-smoker	16	5.95	2.33	0.638
	Smoker	14	5.48	2.97	
Course of disease	Stable	10	4.87	2.18	0.205
	Progressive	20	6.16	2.75	
Type of disease	Classical	29	5.81	2.62	0.358
	Hypertrophic	1	3.30	-	
Distribution of lesions	Generalized	18	6.26	2.99	0.647#
	Generalized + oral	6	4.65	1.34	
	UL	2	6.36	3.32	
	LL	3	4.93	2.03	
	UL + LL	1	3.90	-	

Data are number (n), mean and standard deviation (SD).
*Independent-samoles t-test unless otherwise indicated.
#One-way analysis of varianc (ANOVA).

Age and sex of the control group:

Thirty healthy subjects were taken as a control group. They were completely free on history taking and on clinical examination. The age of the control group ranged between 23-56 years old with mean value of 37 ± 9 years. Sixteen controls (53.3%) were males and 14 were females (46.6%), as shown in Table (2).

Table (2): Comparison of LP cases and controls

Variable	LP (n=30)	Control (n=30)	P-value
Gender (M/F)	28/2	16/14	0.001*
Age (years)	39 ± 14	37 ± 9	0.488#
SOCS3	5.73 ± 2.61	1.03 ± 0.06	<0.001#

Data are ratio or mean ± SD.   *Fisher's exact test.   #Independent-samples t-test

Course of the disease: Regarding course, 10 (33.3%) cases had stable course, 20 (66.7%) cases had progressive course, and 15 cases (50.0%) showed remission and exacerbation, and 19 cases (30.0%) had a progressive course, as shown in table (1).
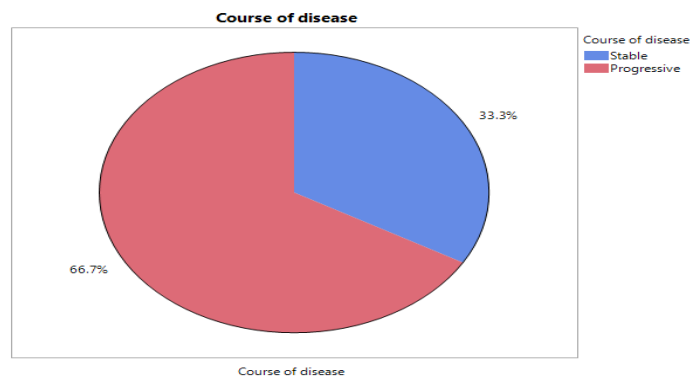

Figure (1): *Proportion of cases with stable or progressive LP*

Distribution of the disease in studied group: Regarding distribution of the disease, 18 cases (60.0%) had generalised distribution, 6 cases (20.0%) had generalised and oral ditribution, 2 cases (6.2%) had upper limb distribution and 3 cases (10.0%) had lower limb distribution and 1 case (3.3%) had both upper and lower limb distribution as shown in figure (2).
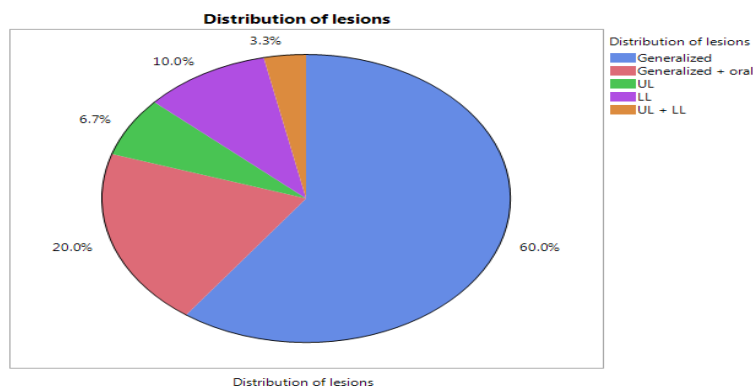

Figure (2): *Distribution of lesions in cases of LP*

Smoking: Regarding smoking, 14 cases (46.7%) were smokers and 16 cases (53.3%) were non-smokers, as shown in figure (3).
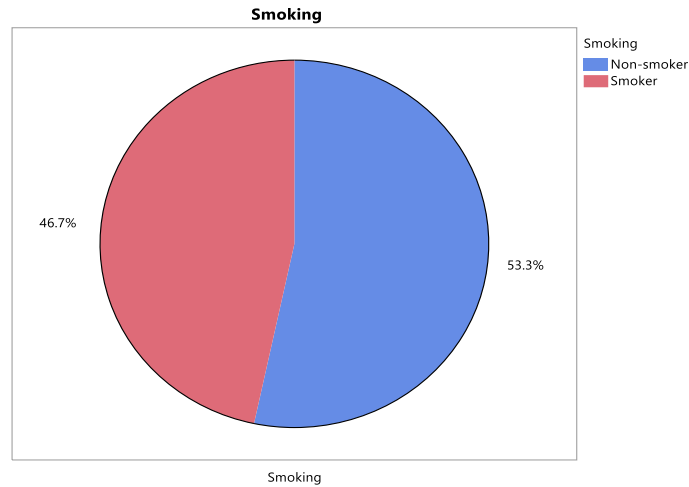
Figure (3): *Proportion of smokers and non-smokers among cases of LP*

Laboratory characteristics of the patients:

Expression of SOCS3 level among the study group : The expression of SOCS3 level in cases reported a mean value of (5.73 ± 2.61). While in controls, it reported a mean value of (1.03 ± 0.06). The expression of SOCS3 was significantly higher in cases group rather than in the control one, with p value (<0.001), as shown in figure (4).
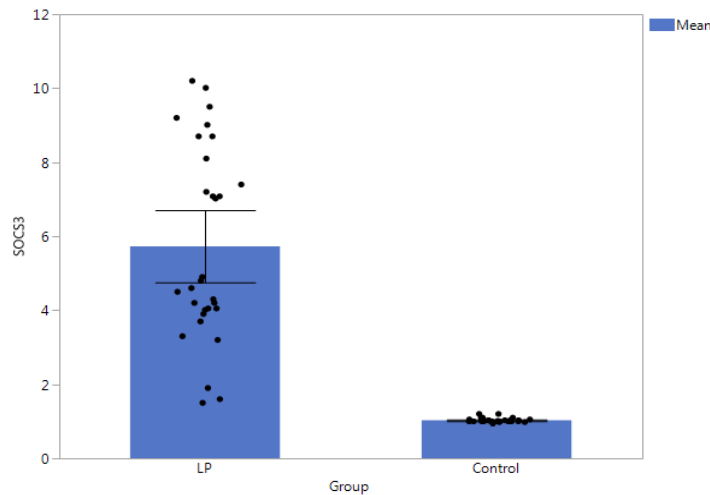

Figure (4): Mean SOCS3 expression in cases of LP and controls. Error bars represent the standard error (SE). Dot markers represent individual observations.

Correlation between SOCS3 level in cases of LP:

The inter relationship between LP cases as regarding SOCS3 level shows P- value of 0.994 in the age variable and 0.790 with the duration of the disease as shown in table (3). The distribution of SOCS3 level in cases with LP ranges but all give elevated levels as shown in figure (5).

Table (3): Correlation between SOCS3 and numerical variables in cases of LP

		Variable	
	Correlation coefficient	Age	Duration of disease
SOCS3	Pearson r	0.001	0.051
	P-value	0.994	0.790
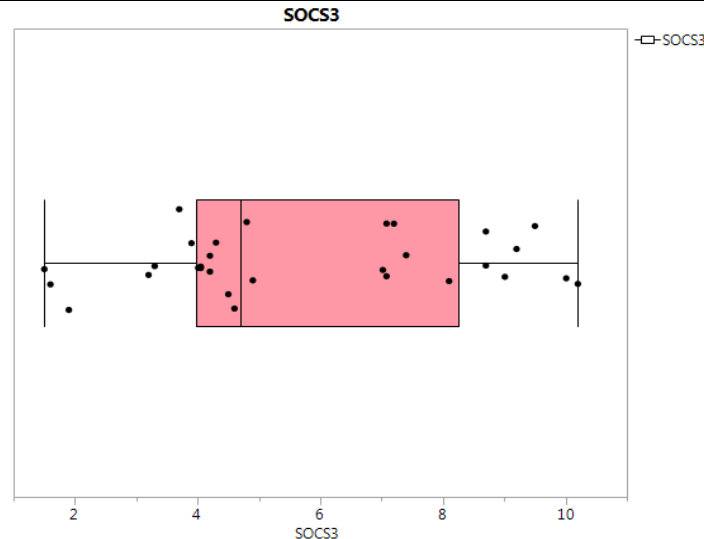
Figure (5): Box plot illustrating the distribution of SOCS3 among LP cases. Box represents the interquartile range. Line inside the box represents the median. Whiskers represent minimum and maximum values excluding outliers (dots).

5. Discussion

The present study aimed to detect the expression of SOCS3 in lichen planus of diseased patients as compared to normal control persons.

This study was a case control study included 30 Lichen planus patients as cases (chosen from outpatients attending dermatology department at Beni-Suef university hospital during the period from January 2019 to July 2019) and 30 normal as controls. The age of LP cases ranged from 21 to 64 years with mean age of 39 years, while the age of the control group ranged between 23-56 years old with mean value of 37 ± 9 years.

Our study agrees with Wang and van der Waal [6] who found that LP mostly affects the middle-aged population. Also, 28 cases (93.33%) were males (mean was 5.68, SD was 2.57) and 2 cases (6.66%) were females (mean was 6.40, SD was 4.38) and P-value 0.714.

Our results agree with Anbar et al. [7], as there were (56 %) males and (44 %) females, and disagree with Omal et al. [8] where (43.9%) were males and (56%) females.

Results of the present study showed that (53.3%) of the LP cases were non–smokers while (46.7%) were smokers. (93.33%) were males; (6.67%) were females.

As regard smoking, our results agree with Cassol-Spanemberg et al. [9] who found that (73.4%) of patients were non-smokers; (26.6 %) of patients smoked, also Sadr Eshkevari et al. [10] found that only (37.1%) of the participants were smokers.

Regarding the course of the disease, (66.7%) of LP cases had progressive disease course while (33.3%) had stable disease course. This agrees with Sangeetha and Victor [11] and Chainani et al. [12] who stated that the LP of the skin is considered to have an acute progression and also a spontaneous remission.

In the present study, the disease duration was 11 ± 11 (1 − 48). In Arora et al. [13] study, duration of lesions ranged from 20 days to 34 months (mean 6.4 months). In Anbar et al. [7] study, the duration of the disease varied from 1 month to 3 years.

Regarding type of the disease, 29 cases (96.7%) had the classical type, whereas 1 case (30.3%) had the hypertrophic type. Our results agree with Arora et al. [13], where classical LP was the most common clinical variant, followed by hypertrophic lichen planus. Also, Hashba et al. [14] found that the most common morphologic type of skin LP was classic LP in (78.9%) patients. On the other hand, Anbar et al. [7] found that (30 %) had classic LP.

Regarding distribution of the disease, 18 cases (60.0%) had generalized distribution, 6 cases (20.0%) had generalized and oral distribution, 2 cases (6.2%) had upper limb distribution and 3 cases (10.0%) had lower limb distribution and 1 case (3.3%) had both upper and lower limb distribution.

In contrast to our study, Khondker et al. [15] stated that lesions were highest (83.33%) in upper limbs, next lower limbs, trunk, oral mucosa.

In our study we found that expression of SOCS3 in lesional skin of LP was significant higher as compared to normal control, where SOCS3 level in cases reported a mean value of (5.73 ± 2.61). While in controls, it reported a mean value of (1.03 ± 0.06) with P- value <0.001. This comes in agreement with Yoshimura et al. [5] who studied its level in oral lichen planus.

In the current study, there was non-significant relation between SOCS3 and duration of the disease, patient's age, course of the neither disease nor type of the disease. Frequent methylation of the SOCS3 gene promoter, theoretically resulting in cytokines expression increase, might be associated with the etiological mechanism of Lichen planus Yoshimura et al. [5].

6. Conclusion

To the best of our knowledge, no similar study has been conducted among the Egyptian population. Our results were unique in studying the role of SOCS3 in skin form of LP. SOCS3 has perfect value for discrimination between LP cases and controls; there was highly statically significant relation between cases and control regarding SOCS3. There were strong relation

between SOCS3 and gender; smoking; and distribution of lesions.

7. Recommendations

This study was limited to a narrow set of samples encompassing patients and controls. In further research, it is expected that an expanded set of datasets be analyzed in order to define the correlation of SOCS3 with other clinical subtypes of LP. Further research is also required in order to study the possible role of SOCS3 in modern therapeutic management of cutaneous LP.

8. References

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