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EXPRESSION OF CYSTATIN SA IN ORAL LICHEN PLANUS

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ABSTRACT

Objectives: identify the diagnostic accuracy of tissue Cystatin (SA) in diagnosis of oral lichen planus

Methodology: The present study included 58 Patients divided into 2 groups, group I: 29 healthy control patients and group II: 29 Patients clinically diagnosed with oral lichen planus. Tissue biopsy was taken and the production of tissue cystatin SA was determined by enzyme immunoassay. We described and compared the protein profiles (Cystatin SA) in the tissues, from patients who presented with oral lichen planus and from healthy controls. Data were presented as mean and standard deviation values and were analyzed using independent t-test. Correlations were analyzed using Spearman's rank order correlation coefficient.

Results: The Oral Lichen Planus group had a significantly higher Cystatin-SA level than the control group ($p<0.001$). The mean value of the cystatin SA in the Oral Lichen Planus group was 19.68±3.69, whereas the mean value of the cystatin SA in the control group was 7.22±1.6, suggesting a role for the cystatin SA in the diagnosi of Oral Lichen Planus.

Conclusions: Cystatin-SA expression in Oral Lichen Planus patients was more outstanding than in healthy control subjects.

KEY WORDS: Biopsy, autoimmune disease, immunoassay

INTRODUCTION

Lichen planus is a disease which affects the stratified squamous epithelia. It is a chronic mucocutaneous, immunological disease with some clinical manifestations. The oral mucosa is usually involved and sometimes it is the only site of involvement [1]. It affects buccal, lingual, gingival and labial mucosa[2]. Clinical forms of Oral Lichen Planus: reticular, papular, plaque-like, atrophic (erythematous), erosive-ulcerative and bullous-erosive. [3] Presence of Wickham white striae is characteristic feature of OLP [4]. Histopathology of OLP shows hyperkeratosis, acanthosis or epithelial atrophy,lymphocytic infiltration in the superficial lamina propria. civatte bodies are present in the superficial lamina propria and in the basal cell layer[5].

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The family of cystatin have many domains containing proteins, found in many organisms and humans. Human type cystatins include cystatin C, D, S, SA and N.

Cystatin-SA is a secreted thiol protease inhibitor which is found in a variety of human tissues, fluids and secretions [6].

Study design

The present study is a diagnostic test accuracy study. The study included 29 patients with oral lichen planus and 29 healthy subjects. All patients and controls were selected from the clinic of Oral Medicine and Periodontology, Faculty of Dentistry, Cairo University. The study protocol was registered on the registry site clinicaltrials.gov under identifier. The study was performed in the period from December2020 till December 2021.

The sample size was calculated according to the mean Log and confidence interval of TIMP-1). Expression previously reported by Rubaci et al. (2012) [9], 56 subjects which was divided into 2 groups (28 subjects per group) Sample size calculation was performed using G* power version 3.1.9.2.

MATERIALS AND METHODS

The present study included 58 Patients divided into 2 groups, group I: 29 healthy control patients and group II: 29 Patients clinically diagnosed with oral lichen planus, Tissue biopsy was taken and the production of tissue cystatin SA was determined by enzyme immunoassay. We described and compared the protein profiles (Cystatin SA) in the tissues, from patients who presented with oral lichen planus and from healthy controls. Data were presented as mean and standard deviation values and were analyzed using independent t-test. Correlations were analyzed using Spearman's rank order correlation coefficient

Inclusion criteria:

- Males and Females
- Age: from 20 to 70 years
- Patients with good oral hygiene measures and ability to follow up
- Patients who are diagnosed with OLP
- Patients who accepted to sign agreement consent

Exclusion criteria

- patients receiving drug therapy systemically or topically within the last 3 months before the study, that may alter tissue expression of inflammatory mediators
- Patients on natural products therapy like green tea
- Patients with malignancy
- Pregnant or Lactating females
- Vulnerable groups as prisoners
- Smokers

Statistical analysis

Data were presented as frequencies and percentages and were analyzed using Fisher's exact test. Numerical data were tested for normality using Shapiro-Wilk test. Data showed parametric distribution, so presented as mean and standard deviation values and were analyzed using an independent t-test and one way ANOVA. Correlations were analyzed using Spearman's rank order correlation

Coefficient ROC curve was constructed to determine the diagnostic accuracy of Cystatin SA in the detection of oral lichen planus. The ROC curve was tested for statistical significance using z-test. The significance level was set at $p \leq 0.05$ for all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Window

RESULTS

Table (1) characteristics of control and diseased groups. The mean ± SD age of control and diseased groups were 55.73 ± 8.24 and 56.72 ± 7.30 years respectively. There was no significant difference between groups in age and sex distribution ($p > 0.05$). About 24 (82.2%) subjects of diseased group had erosive lichen planus and 5 (17.2%) subjects had Reticular lichen planus

Comparison of Cystatin-SA between control and diseased groups

The mean ± SD of Cystatin-SA of control and diseased groups were 7.22 ±1.6 and 19.68 ± 3.69 respectively. The diseased group had a significantly higher Cystatin-SA level than the control group ($p < 0.001$). (Table 2)

Comparison of Cystatin-SA between control, reticular and erosive groups

The mean ± SD of Cystatin-SA of control, reticular and erosive groups were 7.22 ± 1.61, 20.80 ± 4.41 and 19.44 ± 3.59 respectively. Cases with reticular lichen planus had a significantly higher level of Cystatin-SA than healthy individuals ($p<0.001$). Cases with erosive lichen planus had a significantly higher level of Cystatin-SA than healthy individuals ($p<0.001$). There was no significant difference in Cystatin-SA between reticular and erosive groups ($p = 0.464$). (Table 3, figure 1)

TABLE (1). Basic characteristics of participants

	Control group (N = 29)	Diseased group (N = 29)	MD	t- value	p-value
Age (years), Mean ± SD	55.73 ± 8.24	56.72 ± 7.30	-0.99	0.484	0.630
Sex, n (%)					
Male	14 (48.3%)	10 (34.5%)		($\chi^2 = 0.639$)	0.424
Female	15 (51.7%)	19 (65.5%)			
Type of lichen planus, n (%)					
Reticular		5 (17.2%)			
Erosive		24 (82.2%)			

TABLE (2). Mean values of Cystatin-SA of control and diseased groups

	Control group (N = 29) mean ± SD	Diseased group (N = 29) mean ± SD	p-value
Cystatin-SA	7.22 ± 1.6	19.68 ± 3.69	0.001

TABLE (3). Mean cystatin-SA of control, reticular and erosive groups

	Control mean ± SD	Reticular mean ± SD	Erosive mean ± SD	p-value*		
				Control vs reticular	Control vs erosive	Reticular vs erosive
Cystatin-SA	7.22 ± 1.61	20.80 ± 4.41	19.44 ± 3.59	0.001	0.001	0. 464

, Data were analyzed using ANOVA

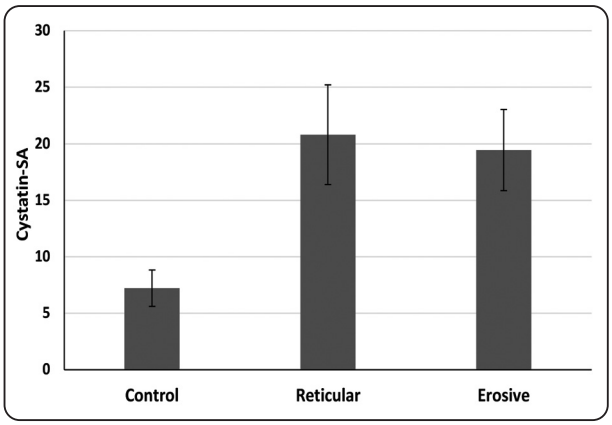

Fig. (1) Mean cystatin-SA of control, reticular and erosive groups

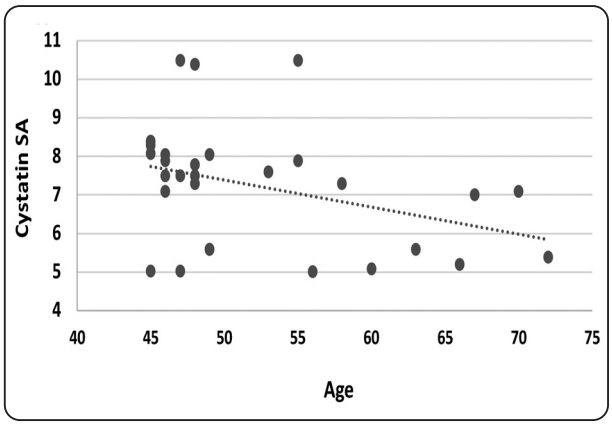

Fig. (2). Scatter plot showing the correlation between Cystatin-SA level and age in the control group.

Comparison of Cystatin-SA between males and females

The mean ± SD of Cystatin-SA of male and female of control group were 7.10±1.23 and 7.33±1.93 and that of diseased group were 19.75±3.55 and 19.64±3.86 respectively. There was no significant difference in Cystatin-SA between male and female ($p > 0.05$). (Table 4).

TABLE (4). Mean values of Cystatin-SA of control and diseased groups

Cystatin-SA	Male	Female	
	mean ± SD	mean ± SD	p-value*
Control	7.10 ± 1.23	7.33 ± 1.93	0.711
Diseased	19.75 ± 3.55	19.64 ± 3.86	0.939

, Data were analyzed using unpaired t-test

Correlation between Cystatin-SA level and age

For the control group, there was a moderate negative significant correlation between Cystatin-SA level and age ($rs = -0.430$, $p = 0.021$), while for diseased group there was no significant correlation ($rs = 0.052$, $p = 0.788$). (Figure 2-3).

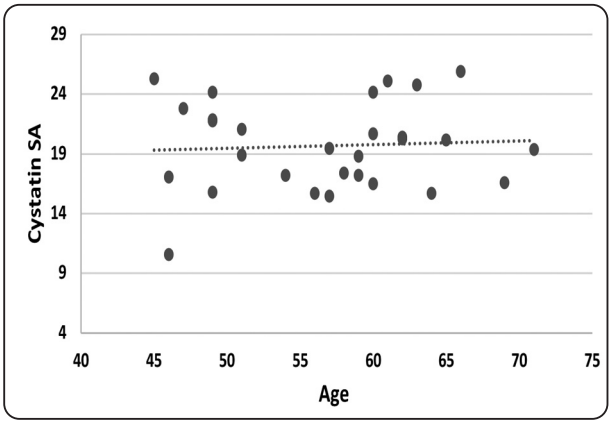

Fig. (3). Scatter plot showing the correlation between Cystatin-SA level and age in the diseased group.

Accuracy of Cystatin SA in diagnosis of oral lichen planus:

The associated sensitivity and specificity of Cystatin SA was 94.05%. The PPV and NPV was 94.05 %. Area under the curve was (.094-1) and was significantly different from (0.5) ($p<0.001$), indicating the higher ability of Cystatin SA levels to distinguish between healthy and diseased subjects. (Table 5) and (figure 4).

TABLE (5). Coordinates of ROC curve analysis at the optimal cut off value:

Parameter	Value [95%CI]
Sensitivity	[94.05]
Specificity	[94.05]
Positive predictive value (PPV)	[94.04]
Negative predictive value (NPV)	[94.05]
Area under the curve (AUC)	[1.00]

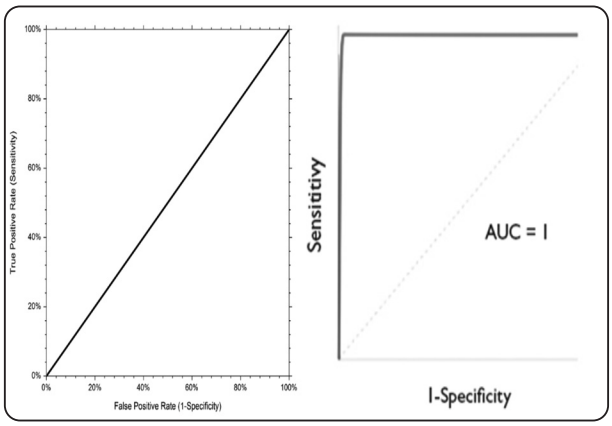

Fig. (4) ROC curve for the accuracy of Cystatin SA in diagnosis of oral lichen planus.

DISCUSSION

The study is a diagnostic test accuracy study. The study included 29 patients with oral lichen planus and 29 control subjects. All patients and controls were selected from the clinic of Oral Medicine, Periodontology and Department, Faculty of Dentistry, Cairo University. The sample size was calculated according to the mean Log and confidence interval of TIMP-1 expression previously reported by **Rubaci et al. (2012)**[9],.

The present study was conducted on 58 subjects, divided into 2 Groups as follows: Group I: This group included 29 healthy normal individuals as controls and GroupII: This group included 29 patients suffering from active oral lichen planus .

Both groups were systemically free as evaluated by the aid of the Medical Complexity Status Classification and Protocol (Glick et al., 2008)[10].

Patients of group II were suffering from the different clinical types of oral lichen planus (OLP) and were selected on the basis of the criteria of Chuang et al(2005)[12].

Diagnosis was confirmed by biopsy. Biopsy was also necessary for detection of the tissue expression of cystatin SA, and its importance as a diagnostic tissue marker for OLP. Tissue biopsy from healthy volunteers (frenectomy, operculectomy, gingivectomy) was taken in the control group.

The study results were in accordance with the results often documented in the literature regarding age and gender distribution, where lichen planus is more common in females and in adults over 40 years old[7,8].

In the present study, the majority of the cases were females with no significant difference (p=0.424)

The OLP group included 10 (34.5%) males and 19(65.5%) females. Whereas the control group included 14 (48.3%) males and 15 (51.7 %) females in the present study, there was no significant difference between ages of the cases in both groups. (p=0.630). The mean age among the OLP group was 56.72±7.30, while the mean age among the control group was 55.73±8.24 .

Regarding the frequency of the type of OLP among the diseased group included in the study, a significantly higher percentage of diseased group had erosive lichen planus (p<0.001) cases had reticular OLP, whereas 24 (82.2%) had erosive OLP 5 (17.2%) .

Regarding the association between Cystatin-SA level and gender, for both groups, there was no significant association between Cystatin-SA level and gender (p>0.05). The mean cystatin .

SA level in the male control group was 7.10±1.23, whereas the mean cystatin level in the female

Control group was7.33±1.93. The mean cystatin level in the male OLP group was 19.75±3.55,

Whereas the mean cystatin level in the female OLP group was 19.64±3.86

Regarding the correlation between Cystatin-SA level and age, for the control group, there was a moderate weak negative correlation between Cystatin-SA level and age (rs=-0.430) (p=0.021), while for diseased group there was no significant correlation (p=0.788).,

Regarding the intergroup comparison of Cystatin-SA level, the diseased group had a significantly higher Cystatin-SA level than the control group (p<0.001). The mean value of the cystatin SA in the OLP group was 19.68±3.69, whereas the mean value of the cystatin SA in the control group was 7.22±1.6. Similar results were obtained by **Bangsuwan et al. (2021)** [11],

Regarding the effect of lichen planus type on Cystatin-SA level, there was no significant difference between different types (p = 0.464). The mean value of the cystatin SA in the reticular group was 20.80±4.41, whereas the mean value of the cystatin SA in the erosive group was 19.44±3.59

1this could be due to the narrow sample size of the reticular group (5 patients, 17.2%) in comparison to the erosive group (24 patients,82.2%).

Regarding the difference in Cystatin-SA levels between healthy individuals and different lichen planus types (reticular and

When the control group was compared to the reticular group, cases with reticular lichen planus had significantly higher level of Cystatin-SA than healthy individuals (p<0.001).

The mean value of the cystatin SA in the control group was7.22±1.61, whereas the mean value of the cystatin SA in the reticular lichen planus group was 20.80±4.41,

When the control group was compared to the erosive group, cases with erosive lichen planus had a significantly higher level of Cystatin-SA than healthy individuals (p<0.001). The mean value of the cystatin SA in the control group was 7.22±1.61, whereas the mean value of the cystatin SA in the erosive group was 19.44±3.59,

Regarding the accuracy of Cystatin SA in diagnosis of oral lichen planus, the optimal cut off value was determined based on Youden index. The associated sensitivity was (94.05%) indicating a high probability of higher Cystatin SA levels when the disease is present. The associated specificity was (94.05%) indicating a high probability of lower Cystatin SA levels

Erosive groups), there was 3 significant difference between the different groups (p=0.001). The mean value of the cystatin SA in the control group was 7.22±1.61,

The positive predictive value (PPV) was (94.05%) indicating high probability of the disease presence when the levels of Cystatin SA levels are high. The negative predictive value (NPV) was (94.05%) indicating high probability of the disease absence when the levels of Cystatin SA levels are low. Area under the curve (AUC) was (1.0) and was significantly different from (0.5) (p<0.001), indicating the higher ability of Cystatin SA levels to distinguish between healthy and diseased subjects.

CONCLUSION

Cystatin-SA expression in OLP patients was more outstanding than in healthy control subjects

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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