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SALIVARY LEVELS OF CATHELICIDIN LL-37 IN PATIENTS WITH ORAL POTENTIALLY MALIGNANT LESIONS, A CASE CONTROL STUDY

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ABSTRACT

Aim: The recognition of practical early diagnostic biomarkers is a cornerstone of improved prevention and treatment of cancer thus the current study estimated salivary level of Cathelicidin LL-37 in patients suffering from potentially malignant lesions and control subjects to corroborate Cathelicidin LL-37 as a diagnostic marker for early detection of potentially malignant diseases and revealing its possible role in carcinogenesis.

Methodology: 45 systemically healthy individuals were subdivided into three groups: Group I: 15 Healthy participants without any oral lesions. Group II: 15 Patients having atrophic/erosive oral lichen planus (OLP). Group III: 15 Patients having oral leukoplakia. Enzyme linked immune-sorbent assay (ELIZA) kit was used to evaluate the level of LL-37 in whole unstimulated salivary samples collected from all participants. To reveal AUC, sensitivity, specificity, and diagnostic accuracy of LL-37 receiver operating curve (ROC) analysis was done.

Results: The highest salivary level of LL-37 was revealed in OLP patients followed by oral leukoplakia patients whereas it was the lowest in healthy controls. ROC analysis exhibited excellent diagnostic accuracy of salivary LL-37 in differentiating both OLP and leukoplakia from control and OLP from leukoplakia.

Conclusions: LL-37 appears to have a potential role in potentially malignant lesions (OLP & leukoplakia). The remarkable diagnostic accuracy of salivary LL-37 in differentiating potentially malignant lesion and healthy control could confirm its utilization as an innovative marker to early diagnose potentially malignant lesions. Salivary LL-37 being non-invasive accurate marker could be a chair-side diagnostic method that detect-potentially malignant lesions.

Clinical relevance: Salivary Cathelicidin LL-37 being non-invasive could serve as a chair-side diagnostic technique for potentially malignant lesions.

KEY WORDS: Oral potentially malignant lesions, saliva, Cathelicidin LL-37

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INTRODUCTION

Oral premalignant diseases (OPMD) are a comprehensive term for an array of lesions that can develop within the oral cavity and comorbid in a variety of patient populations.[1] The most designated premalignant oral lesions are lichen planus, leukoplakia, erythroplakia, and submucous fibrosis.[2-5]

Antimicrobial peptides (AMPs) are essential components of the immune system that can fight a broad spectrum of organisms and transformed or cancerous cells. The mammalian AMPs belong to the defensin and cathelicidin families.[6] Cathelicidin exons 1-4 are located on chromosome 3p21 in the human genome. The exons are transcribed as a single gene, cathelicidin antimicrobial peptide. It translates to an 18 kDa pre-pro-protein, referred to as hCAP18. hCAP18 is the only cathelicidin in humans which is processed through proteolytic cleavage to active cathelicidin LL-37 (37 amino acid residues with diLeucine at the N-terminus). Specific serine proteases like, kallikrein 5, kallikrein 7 and proteinase 3 are involved in this cleavage so it is also called LL-37.[7-10]

Recent studies have verified that human cathelicidin LL-37 could be considered as a chemical defense, it possesses pleotropic functions as eradication of invading pathogens, restoring homeostasis and immunomodulatory functions. LL-37 is implicated in both innate & adaptive immunity as it is capable of chemotactically activate immune cells to the infected tissue in addition to a synergistic activity with different active substances so enhance the differentiation of a lot of immunocompetent cells to proinflammatory ones like monocyte which can be further differentiated into macrophages. Moreover, it regulates the equilibrium between pro- and anti-inflammatory cytokines.[11,12]

Additionally, evolving evidence proposes that LL-37 is also implicated in the regulation of cancer, it showed a complex role in tumorgenicity as LL-37 can be joined with dual aspects of cancer development through several receptors, such as epidermal growth factor receptor (EGFR), FRP2, ERBb2, P2X7, and GAPDH, while on the other hand tumor suppression takes place by interaction with peptide-based factors in addition to cancer membrane components.[9,13]

Therefore, LL-37 may either revealed a protumorigenic action as in cancers of the pancreas, ovary, lung, breast, and prostate, as well as in malignant melanoma and skin squamous cell carcinoma,[13-19] or act as an anti-cancer agent for another cancers lesion as oral squamous cell carcinoma, hematologic malignancy, colon cancer and gastric cancer where LL-37 expression levels were reduced compared to their levels in normal tissues. This divergence may be indorsed to signal regulation, peptide-based factors and host membrane-based factors. Based on these investigations, the effect of LL-37 on cancer is returned to the origin and type of cancer. [10, 20, 21]

The GLOBOCAN database 2018 assessed 354,864 recent cases of oral cancer globally, lip and oral cancers lesions triggered nearly 177,384 deaths in 2018. Diagnosis and management, prediction of cancer metastasis and consideration of the factors that encouraging or hindering cancer cell invasion are essential for people worldwide.[22] So, the present study aimed to determine the salivary level of LL-37 in precancerous lesions for the exploration of the conceivable impact of this peptide on the elaboration of various precancerous lesions in the oral cavity.

SUBJECTS AND METHODS

The present study is an observational prospective study that was accomplished in the Oral Medicine and Periodontology departments, Faculty of Dentistry, Cairo, Fayoum and Ahram Canadian Universities on a section of Egyptian population in the time interval between July to October 2023. Patients were consecutively designated from the outpatient clinics of the departments of Oral

Medicine and Periodontology, Faculty of Dentistry, Cairo, Fayoum and Ahram Canadian Universities. Ethical approval was obtained from the research ethics committee of the institutional review board of Faculty of dentistry Cairo university (Approval number: 63723) and registration of the study on clinicaltrial.gov was done (NCT06219330).

A total number of 45 systemically healthy participants were included in this investigation that were allocated equally into 3 groups: Group I: 15 Healthy participants without any oral lesions. Group II: 15 Patients having atrophic/ erosive oral lichen planus. Group III: 15 Patients having oral leukoplakia. The procedures and purpose of the study were discussed and clarified to all subjects prior to inclusion in the study groups and were then asked to sign written informed consents.

Inclusion criteria

- Both genders with age range 30 - 70 years.
- Participants that sign a written consent after understanding the nature of the study.
- The clinically diagnosed and histologically approved as having oral potentially malignant lesions mainly atrophic/erosive oral lichen planus and oral leukoplakia.

Exclusion criteria

- Systemic diseases as well as pregnant or lactating females.
- Patients currently taking corticosteroids, immunosuppressives drugs, contraceptive pills, or antibiotics.
- Patients diagnosed with any other oral lesions other than oral lichen planus and oral leukoplakia.
- Vulnerable subjects as prisoners, or mentally disabled.

A full comprehensive clinical assessment of all included participants was carried out. The site, type, and dysplastic changes of the lesions were recorded in addition to pain and ulcer scores for oral lichen planus lesions. Histopathological assessment was done. Biopsied lesions from darkly stained areas with toluidine blue were evaluated to confirm the diagnosis of lesions and to assess the degree of dysplasia according to the WHO classification system.[23]

Whole Unstimulated Salivary sampling:

Unstimulated whole salivary samples were collected from all participants enrolled in the study following standard methods.[24] Five ml of saliva was collected from everyone between 09:00 and 11:00 in the morning to avoid circadian rhythm after overnight fast. Patients were asked not to eat, drink (except water) or chew gum. The sample was obtained by tilting the head of the patient forward and expectoration into Eppendorf tube while seated in an upright position. All tubes with salivary samples were given serial numbers and were immediately frozen & stored at (-70 ^{o}C) until the assessment of LL-37. Salivary LL-37 levels were then quantified by using a commercial ELIZA kit.

Detection of Human Antibacterial protein LL-37 in whole unstimulated saliva:

Saliva samples were centrifuged at 4000xg for 10 min then supernatant was separated to be used for determination of LL-37 using ELISA kit. The kit was provided by Bioassay Technology Laboratory Zhejiang, China with Cat.No E2197Hu. This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human LL-37 antibody. LL-37 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human LL-37 Antibody is added and binds to LL-37 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated LL-37 antibody. After incubation unbound Streptavidin-

HRP is washed away during a washing step. Substrate solution is then added, and color develops in proportion to the amount of Human LL-37. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Sample size calculation:

Based upon results of a pilot study conducted on five subjects in each group, the mean and standard deviation (SD) values for LL-37 in saliva were 60.7 (13.1) and 45.8 (3.5) ng/mL in diseased and healthy control groups, respectively. Using alpha (α) level of (5%) and Beta (β) level of (5%) i.e. power = 95% the effect size (d) was 1.55 and the minimum estimated sample size was 12 patients per group. By adding 20% per group to make up for sampling and analysis errors, the estimated sample size was 15 patients per group. Sample size calculation was performed using G*Power Version 3.1.9.2.

Statistical methods:

Data were analyzed using Jamovie 2.3.18 application and were statistically described in terms of mean and standard deviation (±SD), or frequencies (number of cases) and percentages when appropriate. Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests. Homogeneity of variances was tested using Levene's test. For parametric continuous data, the One-way ANOVA followed by Games-Howell Post-Hoc Test (unequal variances) were used to compare more than three independent groups and independent sample t-test was used to compare two independent groups. For non-parametric data, Mann and Whitney test was used to compare two independent groups. For dichotomous data, Chi square test or Fischer's exact tests were used. Pearson's correlation test was used to detect the correlation between the studied marker and clinical scores. A receiver operating characteristic curve (ROC) was created to estimate a preliminary cutoff point for the salivary level and predict diagnostic accuracy of the studied marker. Also, area under the ROC curve (AUC) was calculated according to the rough guide for the accuracy of a diagnostic test classification by the traditional academic point system[25]: (0.90–1.0 = excellent, 0.80–0.90 = good, 0.70–0.80 = fair, 0.60–0.70 = poor, 0.50–0.60 = fail).

RESULTS

The 45 participants consisted of 15 oral leukoplakia patients, 15 OLP patients, and 15 healthy controls. The 15 OLP patients were 9 females and 6 males with mean ages of 53.1±14.9, while the 15 oral leukoplakia patients were 2 females and 13 males with mean ages of 50.8±11.8, whereas the 15 healthy controls were 5 females and 10 males with mean ages of 49.7 ± 14.2. Table (1) shows clinical and demographic data of study participants.

The mean values for salivary LL-37 (ng/ml) are summarized and compared between the study groups in Table (2) which revealed statistically significant difference between all included groups. It is clearly revealed also that the greatest elevation of salivary LL-37 expression level occurred in OLP patients (86.4±8.3) followed by oral leukoplakia patients (63±4.91), whereas it was the lowest in healthy controls (35.2± 4.14). When OPMD were considered as a whole, the mean value of salivary LL-37 was 74.7±13.7, which is apparently significantly higher than that of the control group. Notably, there was no statistically significant difference in the salivary expression level of LL-37 in patients above and below 50 years of age, neither between males and females nor among different grades of dysplasia in the study groups.

TABLE (1) Clinical and Demographic data of study participants.

Variables	Group	Subgroup	Count	Proportion %
Participants	OLP		15	33.3%
	Oral leukoplakia		15	33.3%
	Controls		15	33.3%
Site of lesion	OLP	Buccal Mucosa	8	53.3%
		Tongue	4	26.7%
		Gingiva	2	13.3%
		Labial mucosa	1	6.7%
		Floor of the Mouth	0	0%
	Oral leukoplakia	Buccal Mucosa	10	66.7%
		Tongue	4	26.7%
		Gingiva	0	0%
		Labial mucosa	0	0%
		Floor of the Mouth	1	6.7%
Dysplasia	OLP	No Dysplasia	7	46.7%
		High-Grade	2	13.3%
		Low Grade	6	40%
	Oral leukoplakia	No Dysplasia	5	33.3%
		High-Grade	2	13.3%
		Low Grade	8	53.3%
Sex	OLP	Females	9	60%
		Males	6	40%
	Oral leukoplakia	Females	2	13.3%
		Males	13	86.7%
	Controls	Females	5	33.3%
		Males	10	66.7%
Age groups	OLP	≤50y	7	46.7%
		≥50y	8	53.3%
	Oral leukoplakia	≤50y	6	40%
		≥50y	9	60%
	Controls	≤50y	8	53.3%
		≥50y	7	46.7%

TABLE (2) Salivary Expression levels of LL-37 (ng/ml) according to patient characteristics.

	Groups	Subgroups	N	Salivary LL-37 (ng/ml) (Mean±S.D)	P value
Participants	OLP		15	86.4±8.3	< .001*§
	Oral leukoplakia		15	63±4.91	
	Controls		15	35.2±4.14	
OPMD vs controls	OPMD		30	74.7±13.7	< .001*§
	Controls		15	35.2±4.14	
Age	OLP	≤50y	7	87.1±10	0.787*
		≥50y	8	85.9±7.12	
	Oral leukoplakia	≤50y	6	63.3±5.59	0.906***
		≥50y	9	62.6±4.14	
Sex	OLP	Females	9	83.9±8.7	0.154*
		Males	6	90.3±6.57	
	Oral leukoplakia	Females	2	63.8±5.2	1.00***
		Males	13	61.5±3.32	
Site of lesion	OLP	Buccal Mucosa	8	85.3±7.3	N/A
		Tongue	4	84.1±10.1	
		Gingiva	2	97±5.02	
		Labial mucosa	1	83.7	
		Floor of Mouth	0	-	
	Oral leukoplakia	Buccal Mucosa	10	63.8±5.84	N/A
		Tongue	4	61.5±2.03	
		Gingiva	0	-	
		Labial mucosa	0	-	
		Floor of Mouth	1	61.7	
Dysplasia	OLP	No Dysplasia	7	84.1±6.52	0.077**
		High-Grade	2	91.6±1.63	
		Low Grade	6	87.5±11.04	
	Oral leukoplakia	No Dysplasia	5	60.1±10.767	0.07**
		High-Grade	2	74±4.95	
		Low Grade	8	62.1±1.657	

Note: *independent t-test, **ANOVA test an ***Mann& Whitney test §Significant (p<0.05)

The one-way ANOVA results comparing salivary expression levels of LL-37 revealed a statistically significant difference (P< .001) among the three study groups. Accordingly, we performed a post hoc analysis which yielded statistically significant higher elevation of salivary LL-37 in OLP than oral leukoplakia patients (< .001) as well as higher elevation of this marker in OLP and oral leukoplakia patients than healthy controls (< .001 each) as shown in table (3).

TABLE (3) Post Hoc comparisons of LL-37 saliva (ng/ml) between the 3 study groups.

	Groups		Oral leukoplakia	Controls
Salivary LL-37 (ng/ml)	**OLP**	Mean difference	23.4	51.2
		p-value	< .001***	< .001***
	Oral leukoplakia	Mean difference	-	27.8
		p-value	-	< .001***

Games-Howell Post-Hoc Test. Note. * p < .05, ** p < .01, *** p < .001

Furthermore, results revealed a statistically significant correlation between pain scores of OLP and salivary LL-37 (P =0.003), however, there were no statistically significant correlation between ulcers scores of OLP and salivary LL-37 (P =0.341) as shown in table (4).

When the receiver operator curve (ROC) was designed to test the sensitivity and specificity of salivary LL-37 as a potential biomarker for either OLP or oral leukoplakia against the control, the Youden's index indicated that at a cutoff point of (72.3, 59.2) respectively delineating the difference between disease and control showed the highest sensitivity and specificity of 100% for each. The area under the curve (AUC) was 1.00 which is considered an outstanding diagnostic accuracy. As for the sensitivity and specificity of salivary LL-37 as a potential biomarker for OPMD, the Youden's index indicated that at a cutoff point of (59.7) delineating the difference between disease and control showed the highest sensitivity and specificity of 96.67% and 100% respectively. The positive predictive value (PPV) was 100% and negative predictive value (NPV) was 93.75% and the area under the curve (AUC) was 1.00 which is considered an excellent diagnostic accuracy. Furthermore, ROC analysis revealed that the sensitivity and specificity of salivary LL-37 in differentiating OLP and oral leukoplakia lesions were 100% and 93.33% respectively, the Youden's index indicated a cutoff point of (72.3). The positive predictive value (PPV) was 93.75% and negative predictive value (NPV) was 100% and the area under the curve (AUC) was 0.989 which is considered an excellent diagnostic accuracy as shown in table (5) and figure (1).

TABLE (4) Correlation between clinical parameters of OLP and salivary LL-37

Correlation matrix		**Pain scores**	**Ulcers score**
Clinical score (Mean ± SD)		6.8 ± 2.21	3.47 ± 1.19
Salivary LL-37 (ng/ml)	Pearson's r	0.717	0.341
	P-value	0.003*	0.214

Significant (p<0.05)

TABLE (5) Sensitivity and Specificity of salivary LL-37 (ng/ml) in the diagnosis of OLP, oral leukoplakia and OPMD:

Differentiating	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cutoff point	Youden's index	AUC	Metric Score
OLP vs Control	100%	100%	100%	100%	72.3	1.00	1.00	2.00
Leukoplakia vs Control	100%	100%	100%	100%	59.2	1.00	1.00	2.00
OLP vs Leukoplakia	100%	93.33%	93.75%	100%	72.3	0.933	0.989	1.93
OPMD vs Control	96.67%	100%	100%	93.75%	59.7	0.967	1.00	1.97

PPV: Positive Predictive Value, NPV: Negative Predictive Value

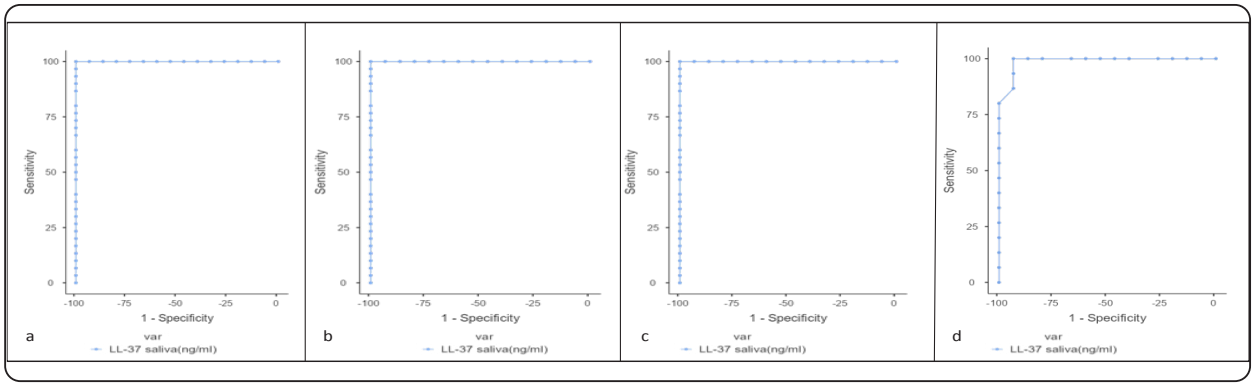

Fig. (1) ROC curve discriminating (a) OLP and Control groups (b) Oral leukoplakia and Control groups (c) OPMD and Control groups (d) OLP and oral leukoplakia groups.

DISCUSSION

Cancer is considered a major world health challenge that now cogitated as the third leading cause of death, unfortunately oral cancers are the sixth utmost common cancer. It is estimated that by 2030 there will be nearly 26 million newfound cancer cases and 17 million cancer-related deaths per year. Recent studies indicate an emergent tendency in oral tumor occurrence and discover that precancerous lesions are one of the principal causes of most oral cancers. Identification and treatment of these cases are critical to preclude patient morbidity and mortality.[9,26,27]

LL-37 is expressed in most tissues and owns a pivotal role in host defense. It acts as mediator of innate immune response against various invading microorganisms, (bacteria, fungi & virus) then performs a bridge to adaptive immunity. As well it is involved in an array of fundamental biological cell activities including migration, proliferation, invasion apoptosis and cell cycle arrest. Additionally, it contributed to tissue homeostasis, angiogenesis, regeneration, re-epithelialization and wound closure. Up till now, LL-37 has been authenticated to show varying tumor-biology-dependent effects on cancerous cells, either as pro tumorigenic or anti-tumorigenic. LL-37 has been detected in saliva and gingival crevicular fluid.[10, 28-32]

The detection of biomarkers from biological body fluids as saliva has the prospect of early diagnosis of several diseases. Saliva encloses proteins, peptides, electrolytes, organic, and inorganic salts secreted by salivary glands in addition to gingival crevicular fluids and mucosal transudates contributions. More than 100 salivary biomarkers have already been recognized in saliva. Saliva as a medium for detection of cancer biomarkers is a promising approach as it is a non-invasive sampling modality in addition to the ease of its collection, furthermore saliva direct contact with oral lesions makes it more precise and hypothetically sensitive screening tool.[33] So this study was performed to investigate the contribution of LL-37 in precancerous lesions via estimation of its level in saliva.

In the present investigation the highest levels of salivary LL-37 were registered among OLP patients followed by leukoplakia patients with the lowest levels found in the control group with statistical significance. Our results regarding salivary LL-37 being significantly higher in OLP than in healthy control were in accordance with our results findings documented in a study by **Okumura**[34] which revealed significantly higher salivary level of LL-37 in OLP than in health subjects. The author found that more hCAP18/LL-37 peptide was expressed in OLP compared to healthy epithelium and was not related to microbial infection. He suggested that the generation of growth factors in inflamed lesion as insulin like growth factor in human keratinocytes induce hCAP18/LL-37 expression.[34] In addition, LL-37 enhances mast cell degranulation, keratinocytes apoptosis and affects release of cytokines like IL-8, all of which were reported as involved factors in pathogenesis of OLP[35], thus supporting our study results concerning the increased level of LL-37 in OLP patients.

Additionally, the present results are consistent with previous study that had estimated salivary level of LL-37 in healthy and OLP patients, their results showed a significantly lowest level of LL-37 in control group, the highest significant level was exhibited in erosive lichen planus which was significantly higher than either control or reticular OLP groups. They suggested that variation in salivary level of LL-37 might coincide with inflammatory diseases with ulcer manifestation presented in targeted role of innate defense mechanisms in soft tissue that could protect against lesion infection and stimulate rapid wound healing.[36] They considered an earlier study that demonstrated an association between salivary concentration of LL-37 and number of monthly occurred ulcers in patients with Behcet's disease as confirmation to their results.[37] Interestingly, both studies reinforced our speculation regarding the role of LL-37 in OLP as our results displayed the highest salivary level in OLP group as an inflammatory clinical condition.

Previous studies have reported that LL-37 peptide is able to augment the cell stiffness of epithelial cells as well as its competence to modulate viscoelastic criteria of the cells and tissues. Moreover, within the oral cavity extracellular matrix stiffness in oral leukoplakia owing to an amplify in collagen regeneration and accumulation in the extracellular matrix is correlated with increased expression of LL-37.[38-40] Thus, the observed results in this study regarding higher levels of LL-37 in our leukoplakia patients compared to healthy controls could be attributed to these postulations. Furthermore, a recent study demonstrated that LL-37 is engrossed in development of oral submucous fibrosis which is also believed as a precancerous lesion that showed many pathological changes including chronic inflammation, local inflammation in the lamina propria or deep connective tissues, disproportionate collagen deposition in the connective tissues below the oral mucosal epithelium, and degenerative changes.[41]

A prominent observation is the alteration in expression of hCAP18/LL-37 in many oral inflammatory diseases that cause raise in the level of LL-37 in saliva, thus could directly promote carcinogenesis as inflammation is considered the

seventh hallmark of tumors.[35,40,42] The mentioned observation could explain the significantly higher levels of salivary LL-37 in OLP than in oral leukoplakia, that was revealed in our study, where inflammation plays a significant role in the pathogenesis of OLP while leukoplakia lacks this inflammatory background. Moreover, our data revealed a significant correlation between pain scores in OLP and salivary LL-37 which may also add evidence to suggested association between inflammation and LL-37.

The link between LL-37 and cancer development returned to the ability of LL-37 to stimulate cell proliferation via reduction of their apoptosis.[40,42] This could explain our results which showed that OPMD, either OLP or leukoplakia, with high grade of dysplasia registered the highest levels of salivary LL-37 followed by lesions with low grade dysplasia then finally the lesions with no dysplastic changes revealed the lowest levels with statistical significance.

As mentioned in literature, an obvious association between severity of inflammation and development of cancer is confirmed,[40,42] which support our findings regarding higher LL-37 salivary levels found in different dysplastic lesions of OLP when compared to their corresponding dysplastic lesions of oral leukoplakia owing to the inflammatory state found in OLP. So, considering the role of this peptide in diagnosis and treatment of precancerous lesion is an important road to be justified.

In the ROC analysis, LL-37 showed powerful diagnostic accuracy between the control and each diseased groups with excellent sensitivity and specificity; thus, LL-37 salivary level could have an outstanding role in diagnosis of oral potentially malignant diseases (OLP & leukoplakia). In addition, this peptide differentiates well between our two diseased groups with excellent diagnostic accuracy, sensitivity, and specificity.

The somewhat small sample size and limited categorization of precancerous lesions with absence of treatment and follow up which could further assist in confirming the contribution of LL-37 in precancerous oral lesions as a marker are considered limitations of our study so further studies considering these limitations are needed in the future. Also, studies comparing LL-37 level between OPMD and oral malignant lesions could be recommended.

Conclusively, literature evidence presented many biological functions of LL-37 owing to its ability to interact with different membrane receptors. Additionally, hCAP18/LL-37 elicits complex responses in various cells, either directly or through the modulation of cellular responses to microbial compounds and other immune mediators.[38]

However up to date the available data on LL-37 impact is inadequate in the aspect of oral cavity. The exploration of the prospective effect of this peptide on the incident of precancerous and cancerous lesions in the oral cavity is still unexplored clearly so our study results shed some light on its contribution in some OPMD for the first time as far as the authors' knowledge with further investigations recommended to determine the exact contribution of this peptide in the pathogenesis of the mentioned diseases which will be of diagnostic and therapeutic potential accordingly decreasing cancer occurrence.

CONCLUSION

LL-37 might contribute to the development of OPMD as revealed in our study results. Moreover, it owes an excellent diagnostic accuracy in differentiation between OPMD and control salivary samples, also it could differentiate between OLP & leukoplakia.

Declarations:

- **Ethics approval and consent to participate:** This study was approved by the research ethics committee of the institutional review board of Faculty of oral and dental medicine Cairo university (Approval number: 63723), following

the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

- **Consent**: All included individuals in this study signed written consent after clarifying to them the steps and aim of the study. All methods were carried out in accordance with relevant guidelines and regulations.
- **Consent for publication:** not applicable.
- **Availability of data and materials:** the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The authors declare that they have no competing interests.
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Authors' contributions:

- S.H contributed to the study design, writing, and submitting the manuscript.
- S.H, N.T and M.Z contributed to data and sample collection.
- S.H, N.T and M.Z contributed to reviewing and revising the manuscript.
- O.S contributed to the determination of salivary LL-37 in samples using ELIZA kit.
- All authors read and approved the final manuscript.

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- **Authors' information:** not applicable
- **Conflict of interest:** Authors of the current investigation declare they have no conflict of interests.
- **Clinical trial registration:** NCT06087042

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