



# Examination of Candida Albicans in Saliva of Iraqi lichen Planus Patients

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**Abstract :** Background: One to two percent of the population suffers from lichen planus, an inflammatory skin disorder that may affect almost every part of the body, including the nails, hair, and mucous membranes. Even though lichen planus may present in a variety of ways, the most common sites of involvement are the skin and the mouth. A superinfection with Candida species can worsen the symptoms of oral lichen planus, particularly in its erosive variants, where one of the most opportunistic mycosis globally is the Candida ssp and the research aims to evaluate the prevalences of candida albicans in lichen planus patients (oral & skin) in comparison to healthy control group. Material and method: The sample consists of 90 subjects divided into 3 groups each of 30 as follow: oral lichen planus group ,skin lichen planus group and control healthy group. Saliva samples obtained from all subjects were cultivated on selective media (SDA) and colony forming unit per milliliter of Candida albicans was estimated after incubation at 37c for 24- 48 h. Results: Results shows the prevalence of Candida albicans as a proportion in "Oral L.P. and healthy control." groups, showed highly significant difference while the proportion in "Oral L.P. and skin L.P. groups, results showed no significant difference and Finally, the proportion in "Skin L.P. and Control" did not reveal any discernible variation . Conclusion: the prevalence of Candida albicans in oral L.P with healthy control "showed highly significant difference while comparing "Oral L.P. and skin L.P." there was no difference. Lastly, the "Skin L.P. and healthy Control", show no differences between them.

**Keywords:** Human saliva, Oral Lichen planus, Skin lichen planus, Candida albicans, SDA.

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## Introduction

Stratified squamous epithelia are often affected by lichen planus (LP) disease, a mucocutaneous condition. The etiology of the disease is complex with histological characteristics more typical in cutaneous lesions than mucosal lesion where ulceration is more noticeable, LP mostly strikes middle-aged adults, especially women in the peri menopausal stage. Lichen planus typically manifests on the skin, mouth, scalp (lichen planopilaris), nails

(lichen unguis), genitalia (penile or vulvar lichen planus) or even under the skin, such as in the oesophagus(1).

The cause of oral lichen planus, a prevalent disease of the oral mucosa, is still unknown despite the presence of several risk factors. Possible etiological reasons for the lesion's eruption and/or a predisposing factor for its emergence include stress factors, which might exacerbate the lesion (2).

The majority of cutaneous types of this chronic inflammatory illness

usually disappear on their own within a year or two, although there are exceptions. mucosal lichen planus and skin hypertrophy represent a potential premalignant condition Because the incidence in those LP variations is around 1% (3).

Purple, polygonal, pruritic papules and plaque are the clinical hallmarks of LP cutaneous lesions. Wickham striae, which are distinctive, very fine, grayish-white lines, often cover the lesion's surface. Most often seen on the insides of the thighs, knees, and flexor surfaces of the limbs as well as the trunk, the initial symptom of the disease is pruritis, mucosal involvement of L.P most often seen on the oral cavity, palate, gums, and tongue, lips, and buccal mucosa, these lesions are asymptomatic and bilaterally or symmetrically distributed usually precede the appearance of cutaneous lesions(4).

*Candida albicans* is the most important fungal opportunistic pathogen, it usually resides as a commensal in the gastrointestinal and genitourinary tracts and in the oral and conjunctival flora (5).

Also *Candida albicans*, may cause a variety of infections including vaginitis, oral thrush, endocarditis, septicemia, skin infections, nail infections, and lung infections (6).

Despite the lack of clarity on the potential link between *Candida* species colonization and OLP, treating OLP lesions infected with *Candida* with antifungal medication alleviates the disease's symptoms. About 8% of healthy people have *Candida albicans* in their oral microbiome. This proportion is much greater in individuals with OLP (7).

The erosive types of OLP might worsen by a superinfection with *Candida*. Nitrosamine and acetaldehyde

are only two of the carcinogenic substances produced by *Candida* metabolism (8).

OLP lesions exposed to known risk factors for oral cancer, such as smoking, alcohol intake, or *Candida* species superinfection, need regular follow up due to increased their potential for malignant transformation (9).

The purpose of this research was to compare the prevalence of *Candida albicans* in the saliva of people with lichen planus to that of healthy controls.

### Materials and Methods

The study conducted in Alkarkh General Hospital/ dermatology department in Baghdad from 30 July ended at 30 December /2023. The subjects in this study included a total (90) individual, 30 of them were oral lichen planus patients and 30 with skin lichen planus patients ,all of them were out patients of dermatological clinic compared with (30) healthy control. All subjects were within the age range 20-65yrs and *candida* colony count was calculated in saliva of those subjects .

Saliva collection was done at specific time on early morning 9A.M-12P.M instruction was given to the patient on a strict no-food, no-hydration regimen for 1 hour before collecting process then all individuals were instructed to spit saliva for 5 minutes without swallowing into a clean plastic container (10).

0.5 ml of saliva is then with down for *candida* detection by making serial dilution of ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ) each dilution was cultivated by transferring 0.1 ml of the sample on the SDA media with addition of gentamycin 5 mg / L for isolation of *Candida albicans* and spread the entire plate using a sterile hockey stick and incubated aerobically at 37 °C for 24-48hrs (11).

*Candida* morphology was examined microscopically by using Gram stain and macroscopically depending on

colony morphology on SDA then *Candida* species isolation and identification confirmed by the use of germ tube (12).

The distinction of *Candida albicans* was based on the Marks of a Colony (color, consistency and topography), Negative colonial trend (color, significant pigment) and morphology at the microscopic level (13).

Isolates of *C. albicans* were typically identified by their ability to form germ tube or chlamydospores under appropriate conditions (14).

### Statistical analysis

The following test was used in this study:

- a. Odds Ratio (OR) with 95% confidence interval: Is a measure of association between an exposure and

an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.

- b. The z-score test: To determine whether there is dissimilarity between two categories that is statistically significant or populations on a single (categorical) attribute, statisticians apply the z-score test for two population proportions.
- c. Receiver Operation Characteristic curve [ROC]: curve and estimating Area, as well as estimating 95% confidence interval, with standard error, asymptotic significant level Receiver Operation Characteristic [ROC] curve.

## Results

**Table(1): Redistribution of the total *Candida* count with reference to two categories responding (Pos. & Neg.) outcomes with comparison's significant.**

Groups	No. & %	Candida Prevalence		Total	C.S. (*) P-value Odds Ratio
		Positive	Negative		
Oral L.P.	No.	21	9	30	C.C. = 0.344 P=0.004 (HS) O.R. = 4.667 95% C.I. (1.571 : 13.866)
	%	70.0%	30.0%	100%	
Control	No.	10	20	30	
	%	33.3%	66.7%	100%	
Total	No.	31	29	60	
	%	51.7%	48.3%	100%	
Skin L.P.	No.	16	14	30	C.C. = 0.198 P=0.118 (NS) O.R. = 2.286 95% C.I. (0.804 : 6.495)
	%	53.3%	46.7%	100%	
Control	No.	10	20	30	
	%	33.3%	66.7%	100%	
Total	No.	26	34	60	
	%	43.3%	56.7%	100%	
Oral L.P.	No.	21	9	30	C.C. = 0.169 P=0.184 (NS) O.R. = 2.042 95% C.I. (0.707 : 5.895)
	%	70.0%	30.0%	100%	
Skin L.P.	No.	16	14	30	
	%	53.3%	46.7%	100%	
Total	No.	37	23	60	
	%	61.7%	38.3%	100%	

(\*) HS: Highly Significant at  $P < 0.01$ ; NS: Non Significant at  $P > 0.05$ .

Results shows that "Oral L.P." group has recorded highly significant relationship at  $P < 0.01$  in light of control group, as well as an odds ratio

indicating that a diseased group may be exposed to *Candida* prevalence about five times compared to control group, as well as preceding prevalence might be

achieved to fourteen times in the studied of sampling population, and rather than no significant relationship are accounted for association between "Skin L.P." group and control at  $P > 0.05$ , but an odds ratio indicating that a diseased group may be exposed to *Candida* prevalence with more than two times compared to control, as well as preceding prevalence might be achieved to six times in the studied of sampling

population, then finally rather than no significant relationship are accounted for association between diseased groups at  $P > 0.05$ , but an odds ratio indicating that "Oral L.P." group may be exposed to *Candida* prevalence with more than two times compared to "Skin L.P." group, as well as preceding prevalence might be achieved to six times in the studied of sampling population

**Table (2): Testing between two proportions among the numbers of *Candida albicans* in "Lichen Planus" patient groups compared to control groups.**

Multiple Comparisons	No. of Responses	Proportion	Z-Score	Sig. Level	C.L. %	C.S.
O.L.P. X S.L.P.	21	0.700	1.3277	0.18352	$\geq 81.684 \%$	NS
O.L.P. X Control	16	0.533	2.8418	0.00452	$\geq 99.548 \%$	HS
S.L.P. X Control	10	0.333	1.5632	0.11876	$\geq 88.124 \%$	NS

(\*) HS: Highly Sig. at  $P > 0.01$ ; NS: Non Sig. at  $P > 0.05$ .

Statistical analysis did not reveal a statistically significant difference between the "Oral L.P. and skin L.P." percentage testing groups ( $P > 0.05$ ) where z test shows 1.328 where us for testing between proportion of "Oral L.P. and the "Control" " groups, a statistically significant difference was found at  $P < 0.01$  and z test equal 2.842.

Finally, with reference to testing between proportion of "Skin L.P. and

Control" the findings revealed no significant differences ( $P > 0.05$ ) and z test equal 1.563.

As shown in the table above the number of patients infected with *Candida albicans* was 21 out of 30 in oral L.P group (70%), in skin L.P group 16 from 30 (53%), and 10 from 30 (33.3) in healthy control group.

**Table(3) : ROC curve of *Candida* Prevalence Marker concerning of Patients Groups & Control along probable combinations pairwise comparisons.**

Markers	Cutoff Pint	Sen.	Spec.	Area	Std. Error	Asymp. Sig.	Asymp. 95% C.I.	
							L.b.	U.b.
Oral L.P. vs. Control	0.500	0.700	0.667	0.683	0.070	0.015	0.546	0.820
Skin L.P. vs. Control	0.500	0.533	0.667	0.600	0.074	0.183	0.456	0.744
Skin L.P. vs. Oral L.P.	0.500	0.533	0.300	0.417	0.074	0.268	0.438	0.729

(\*) S: Significant at  $P < 0.05$ ; NS: Non Significant at  $P > 0.05$ ; The positive actual state is Positive.

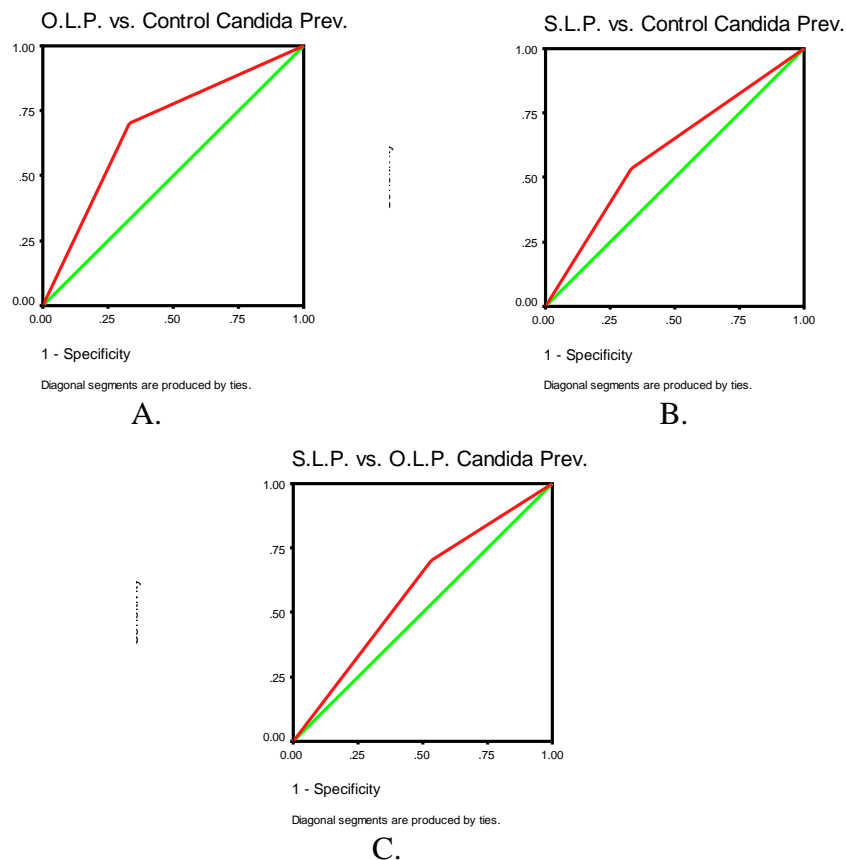
Results shows that *Candida* prevalence are recorded significant area at  $P < 0.05$  under the guideline of control in light of Oral L.P. groups, which was represented by ROC-curve,, while skin L.P. position of patient's group has recorded no significant area under a curve at  $P > 0.05$  under the guideline of control. As well as, area could be arrival

to estimate probability value up to (0.820) in the studied of a diseased sampling population of Oral L.P. patient's group as illustrated by 95% confidence interval of are under the curve –AUC, where the area could be arrival to estimate probability value up to (0.744) in the studied of a diseased sampling population of Skin L.P.

patient's group as illustrated by the 95% confidence interval of area under the curve –AUC.

Finally, no significant of AUC at  $P>0.05$  for the studied marker of Candida prevalence concerning a target line of Oral L.P. position group, under the guideline of Skin L.P. group, as well as, area could be arrival to estimate probability value up to (0.729) in the studied of a diseased sampling population of Oral L.P. patient's group as illustrated by 95% confidence interval of area under the curve –AUC.

Figure (1)(A,B,C) represent graphically ROC curve plots for Candida prevalence regarding of redistribution the total count with reference to two categories responding, such as positive and negative outcomes along all probable combinations pairwise comparisons, patients groups & control under the guideline of Oral L.P. and Skin L.P. groups and control as a baseline group, and between a diseased groups under the guideline of Oral L.P. group vs. Skin L.P. group as a baseline group.



**Figure (1): (A,B,C): ROC Curve plots for Prevalence Marker concerning of Patients Groups & Control along probable combinations pairwise comparisons under a baseline of control and patient's group as the target line.**

## Discussion

In this study with respect to age in oral L.P. group, it is ranged between 40 and 65 years and the mean age:  $(50.83 \pm 6.60)$  years) where in skin L.P. group, the age ranged between 19 and

65 years with a mean age  $(47.37 \pm 11.14)$  years.

This result agree with the mean age of L.P patients in a study done in Baghdad which showed a mean age of 48.3 years (15).

Another study done and the results are consistent with retrospective research on oral lichen planus that included 152 Iraqi patients; the average age of the patients was 46 years, and the most afflicted age group was those in the fifth decayed of life (16).

As well as a Study done in Northern Finland shown that patients' average ages were 54.2 year (17).

### Sex

The present study showed that with respect to sex distribution within L.P groups , a total (20 female 66.7% and 10 male 33.3% found in each L.P group.

These agreed with study who showed a percentage of L.P exposure was 69 % for women and 31 % for men (18).

Another study done on 550 L.P patients have showed a percentage of female equal 76.7% and of male was 23.3% which also coincide with the current study (19).

The present study result showed the prevalence of *Candida albicans* as a proportion comparing oral L.P with healthy control " was highly significant difference between them while comparing "Oral L.P. and skin L.P." Both groups showed no significant variation in terms of the outcomes. Finally, with a proportion of "Skin L.P. and Control" groups, results show no significant differences between the two groups.

Another study who found no statistically significant variation between erosive lichen planus patients and healthy control group considering presence of candida species (20).

Another authors whom conducted a meta-analysis study found that *Candida* species was found in oral lichen planus (OLP) lesions with a frequency of 37%. which estimated about 2½ times more than healthy controls and those meta-analysis included Twenty-four studies (21).

Clinically the classification of OLP is the Non-erosive and erosive forms. The first OLP forms are usually asymptomatic needs just routinely checking up on the lesions. Erosive OLP forms, on the other hand is symptomatic, necessitating therapy and regular checkups because it has a potential for malignancy ,it was shown that detection of candida species significantly greater in erosive OLP than in non-erosive types, because firstly mucosal integrity will breakdown as superficial ulceration presented and that will facilitating the colonization of the oral mucosa by *Candida* species, and second, the modification of the oral microenvironment by erosive OLP lesions, allowing adaptation of certain *Candida* species to thrive in this environment (22).

Rezazadeh et al, found that there is no significant difference in the prevalence of *Candida* between people with dysplastic and non-dysplastic lichen planus, and the incidence is not elevated compared to healthy persons (23).

Another study done who came to conclusion that it should be noted that about patients at risk of *Candida* superinfection may include those with ulcerative OLP/OLR, and who use a topical steroid applications daily, and the one-third of patients with OLP/OLR who receive corticosteroid treatment will have candida superinfection (24).

Although several researches reported that OLP attracts *C. albicans* in the lesions. Because they consider that candida were the primary pathogen of OLP Since OLP can be effectively treated by improving oral hygiene, These clinical considerations suggest that treating E-OLP with application of corticosteroids, followed by or concurrently addressing dental and hygiene issues, is more likely to have a

therapeutic impact . On other hand neglected patients' with poor oral hygiene are contributing to prolong healing times and OLP recurrences . Actually, the clinical experience has shown that improving oral hygiene and treating dental problems—which often involve numerous fungi and *Candida*—is the most effective and accurate way to cure oral lichen planus (OLP) compared to other therapies like immune drugs or herbal products (25).

A study done by group of authors came to conclusion that a prevalence pattern of *Candida* species and oral candidiasis in patients have a mucosal lesion was in order as follow: The most common oral mucosal diseases were Xerostomia, oral lichen planus, chronic ulceration of the mouth, burning sensations in the mouth, stomatitis caused by anemia (26).

Several factors, such as immunosuppression, medications, malnutrition, cancer, age, and poor oral hygiene, can increase the colonization of *Candida albicans* (27).

### Conclusion

Microbiological finding of this study showed highly significant difference in the prevalence of *Candida albicans* between oral L.P with healthy control whereas regarding the prevalence of "Oral L.P. and skin L.P." groups, results showed no significant difference and also between "Skin L.P. and Control" groups results showed no significant difference.

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