Int. j. adv. multidisc. res. stud. 2023; 3(3):97-101

Received: 19-03-2023
Accepted: 29-04-2023

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Changes in Biological Behavior of *Candida*-Infected Oral Leukoplakias: A Meta-Analysis

[1] Rodriguez-Archilla Alberto, [2] Santos-Moron Anabel

[1, 2] Department of Stomatology, Oral Medicine Unit, Faculty of Odontology, University of Granada, Granada, Spain

Corresponding Author: **Rodriguez-Archilla Alberto**

Abstract

Background: Oral leukoplakia is the most common potentially malignant oral lesion. Candida superinfection changes its biological behavior.

Objective: To assess Candida infection of oral leukoplakias and its influence on the biological behavior of these lesions.

Search and selection methods: A search for studies on Candida infection and oral leukoplakia was conducted in the following databases: PubMed (MEDLINE, Cochrane Library), Web of Science (WoS), and Scopus.

Data analysis: The estimation of the proportion was carried out with the generic inverse variance method, using the standard error of the proportion (SE) and 95% confidence intervals (CI95%). For continuous outcomes, the estimates of effects of an intervention were expressed as mean differences (MD) using the inverse variance (IV) method, and for dichotomous outcomes, the estimates of effects of an intervention were expressed as odds ratios (OR) using Mantel-Haenszel (M-H) method all with CI95%.

Results: 17 studies that considered 2,100 oral leukoplakias were included in this meta-analysis. The mean prevalence of Candida-infected oral leukoplakias was 35%. Non-homogeneous oral leukoplakias quadruple the risk of being infected by Candida ($p<0.001$). Regarding epithelial dysplasia, Candida-infected oral leukoplakias were 2.47 times more likely to be dysplastic lesions ($p<0.001$) and 3.83 times more likely to show moderate-severe dysplasia ($p=0.03$). However, age, gender or mild epithelial dysplasia were factors with no relevant influence on Candida infection of oral leukoplakias ($p>0.05$).

Keywords: Candida, Leukoplakia, Oral, Precancerous Conditions, Prognosis

1. Introduction

Oral leukoplakia is defined as "*a predominantly white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer*" [1]. Oral leukoplakia has an estimated prevalence in the general population between 1.5% and 4.3%. Oral leukoplakia is much more common in tobacco users (smoked and smokeless). Other risk factors include alcohol consumption and Candida or human papillomavirus (HPV) infection. Oral leukoplakia is the most common oral potentially malignant disorder with a malignant transformation rate of around 5%. There are two main oral leukoplakia clinical forms: homogeneous and non-homogeneous. The distinction between the two is exclusively clinical, based on the color of the lesion and its morphological characteristics. Non-homogeneous oral leukoplakias are those with the greatest potential for malignant transformation and usually show histopathological changes that vary from epithelial dysplasia to invasive carcinoma [2]. The involvement of Candida species in the etiology or progression of oral leukoplakia is the subject of constant study. Candida species can produce nitrosamines, a well-known carcinogen that may change the biological behavior of oral leukoplakias. The percentage of oral leukoplakias superinfected by Candida species varies between 13%-82%, with non-homogeneous leukoplakias (erythroleukoplakias, speckled or nodular ones, and verrucous forms) being the most infected [3]. Candidal superinfection of oral leukoplakias leads to the more frequent observation of non-homogeneous clinical forms and dysplastic lesions that have a higher malignancy risk. Treatment of this candidal superinfection does not lead to the resolution of the lesion, although it could cause the transformation of the high-risk, non-homogeneous oral leukoplakia into a low-risk, homogeneous clinical form. For this reason, this fungal superinfection is considered a relevant pro-oncogenic factor [4]. This study aimed to assess the candidal infection of oral leukoplakias and its influence on the biological behavior of these lesions.

2. Material and Methods

All the research steps (search, study selection, and data extraction) were developed independently by both authors (ARA and ASM). Potential discrepancies in the article selection were resolved by consensus.

2.1 Search Strategy

A search for studies on *Candida* species and oral leukoplakia up to December 2022 was performed in the following databases: PubMed (MEDLINE, Cochrane Library), Web of Science (WoS), and Scopus. Search strategies included a combination of Medical Subjects Headings (MeSH) and free text terms. Search terms were as follows: ("Candida"[MeSH Terms] OR "Candida"[All Fields]) AND ("leukoplakia, oral"[MeSH Terms] OR "oral leukoplakia"[All Fields]); "Candida" AND "oral leukoplakia"; TITLE-ABS-KEY (("Candida") AND ("oral leukoplakia")). The inclusion criteria were as follows: a) all types of articles related to our purpose, b) articles without relevant risk of bias (score ≥6 stars on the Newcastle-Ottawa methodological quality assessment scale), [5] and c) articles written in any language and with no restrictions on publication date. The exclusion criteria were: a) articles with no full-text availability, b) articles with no clinical data, and d) studies with non-usable data.

2.2 Data Extraction

The prevalence of *Candida* species detection in oral leukoplakias was determined. Other analyzed parameters were age (in years), gender (male/female), clinical form of oral leukoplakia (homogeneous/non-homogeneous), the presence of epithelial dysplasia (yes/no), and the grade of dysplasia (mild/moderate-severe).

2.3 Assessment of Methodological Quality

The methodological quality of the articles was screened using the Newcastle-Ottawa (NOS) methodological quality assessment scale [5] composed of eight items that evaluate three dimensions (selection, comparability, and exposure). Considering the score obtained, the studies are classified as high quality (≥7 stars), moderate quality (4-6 stars), and low quality (1-3 stars).

2.4 Statistic Analysis

For the meta-analysis, data were processed with the RevMan 5.4 program (The Cochrane Collaboration, Oxford, UK). The proportion (P) was calculated by dividing the number of cases (n) by the total population (N). The proportion estimation was carried out with the generic inverse variance method, using the standard error of the proportion (SE) and confidence intervals of 95% (95%CI). The standard error of the proportion (SE) was obtained according to the formula SQRT (P * (1-P) / N). For continuous outcomes, the inverse variance (IV) for the mean difference (MD), and for dichotomous outcomes, the odds ratio (OR) with the Mantel-Haenszel Chi-square formula (M-H) was used, both with 95%CI. Heterogeneity was determined according to the Higgins statistic (I^2). In cases of high heterogeneity (I^2>50%), the random-effects model was applied. A P-value below 0.05 was considered the minimum level of significance.

3. Results

3.1 Study Selection

The literature search yielded 245 articles (119 in PubMed, 73 in WoS, and 53 in Scopus) between the years 1965 and 2021, 51 of them duplicates, leaving 194 articles for eligibility. 177 articles were excluded due to: a) articles with no full-text availability (n=46), b) articles without clinical data (n=39), and c) studies with non-usable data (n=92). Finally, 17 studies were included in this meta-analysis (Figure 1).

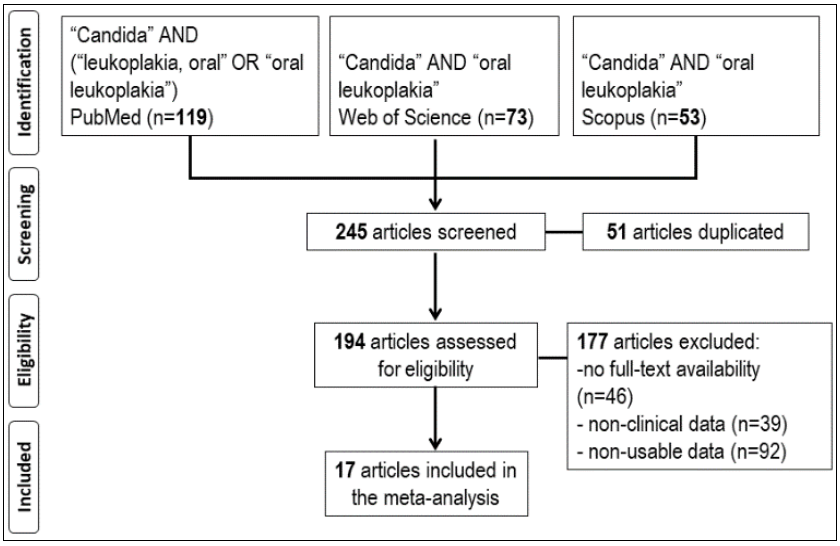

Fig 1: Study selection flowchart.

Table 1 presents the main descriptive characteristics and the methodological quality according to the NOS scale of the seventeen studies [6-22] included in this meta-analysis. A total of 2,100 patients with oral leukoplakia (57.8% males and 42.2% females) were considered. By *Candida* species detection, 22.1% of oral leukoplakias were infected by *Candida* species while 77.9% were not. The studies were conducted in the following countries: India (6 studies), Denmark (3 studies), the United Kingdom (1 study), Ireland (1 study), Spain (1 study), Iraq (1 study), Sri Lanka (1 study), Malaysia (1 study), China (1 study), and Colombia (1 study). According to the NOS quality scale, eight articles (47.0%) had 6 stars, seven articles (41.2%) got 7 stars, and two articles (11.8%) reached 8 stars.

Table 1: Characteristics and methodological quality evaluation of the seventeen articles included in this meta-analysis.

Study, year	Country	Study population	Oral leukoplakia (infected, non-infected)	Other parameters analyzed	NOS
Jepsen, 1965 [6]	Denmark	48 pat (39M, 9F; 28-74y)	35 (54.7%) CL 29 (45.3%) NCL	Clinical type, *Candida* species, locations, histopathological features.	7
Cawson, 1966 [7]	UK	15 pat (11M, 4F; 27-70y)*	15 (10.9%) CL 123 (89.1%) NCL	Locations, duration, histopathological features.	7
Renstrup, 1970 [8]	Denmark	235 pat (154M, 81F; 25-90y)	55 (23.4%) CL 180 (76.6%) NCL	Clinical type, histopathological features.	7
Roed-Petersen, 1970 [9]	Denmark	226 pat (na, na; na)	30 (30.6%) CL 68 (69.4%) NCL	Histopathological features.	6
Daftary, 1972 [10]	India	682 pat (na, na; na)	48 (6.8%) CL 675 (93.2%) NCL	Clinical type, locations, histopathological features.	6
Lipperheide, 1996 [11]	Spain	35 pat (16M, 19F; na)	19 (54.3%) CL 17 (45.7%) NCL	*Candida* species.	6
Al-Hussain, 2006 [12]	Iraq	53 pat (2M, 51F; 40-70y)	37 (69.8%) CL 16 (30.2%) NCL	*Candida* species, locations.	7
Bhavasar, 2010 [13]	India	15 pat (na, na; na)	7 (46.7%) CL 8 (53.3%) NCL	*Candida* detection methods.	6
Dany, 2011 [14]	India	30 pat (na, na; na)	11 (36.7%) CL 19 (63.3%) NCL	Histopathological features.	6
Abdulrahim, 2013 [15]	Ireland	78 pat (39M, 39F; na)	35 (54.7%) CL 29 (45.3%) NCL	Clinical type, *Candida* species, locations, histopathological features, tobacco, denture wearer.	7
Wu, 2013 [16]	China	396 pat (197M, 199F; 7-90y)	59 (15.9%) CL 337 (84.1%) NCL	Clinical type, locations, histopathological features.	8
Bakri, 2014 [17]	Malaysia	28 pat (na, na; na)	12 (42.8%) CL 16 (57.2%) NCL	Histopathological features.	6
Sarkar, 2014 [18]	India	40 pat (35M, 5F; na)	19 (47.5%) CL 21 (52.5%) NCL	Clinical type, histopathological features, tobacco, alcohol.	6
Dilhari, 2016 [19]	Sri Lanka	80 pat (64M, 16F; 32-86y)	38 (47.5%) CL 42 (52.5%) NCL	Tobacco, alcohol.	7
Sankari, 2019 [20]	India	48 pat (na, na; na)	22 (45.8%) CL 26 (54.2%) NCL	Other oral lesions.	6
Erira, 2021[21]	Colombia	30 pat (7M, 23F; 30-90y)	7 (23.3%) CL 23 (76.7%) NCL	Clinical type, locations, histopathological features, tobacco.	7
Gupta, 2021 [22]	India	61 pat (55M, 6F; 25-65y)	23 (37.7%) CL 38 (62.3%) NCL	Clinical type, *Candida* species, locations, histopathological features, tobacco, alcohol.	8

UK: United Kingdom; **pat:** patients with oral leukoplakia; **M:** male; **F:** female; **y:** age range in years; **CL**: *Candida*-infected oral leukoplakias; **NCL:** non-*Candida* oral leukoplakias; **na:** data not available; **NOS:** Newcastle-Ottawa methodological quality scale; *****only data from patients with *Candida* infected oral leukoplakias.

3.2 Detection of *Candida* Species in Oral Leukoplakias

Seventeen studies [6-22] that included 2,139 oral leukoplakias (Figure 2), found an estimated prevalence of *Candida* species detection of 35% (95%CI: 27% to 42%) with a high heterogeneity between studies (I^2: 95%). *Candida* detection rates by study ranged from the maximum frequency of 80% (95%CI: 60% to 100%) [13] to the minimum of 7% (95%CI: 5% to 9%) [10].

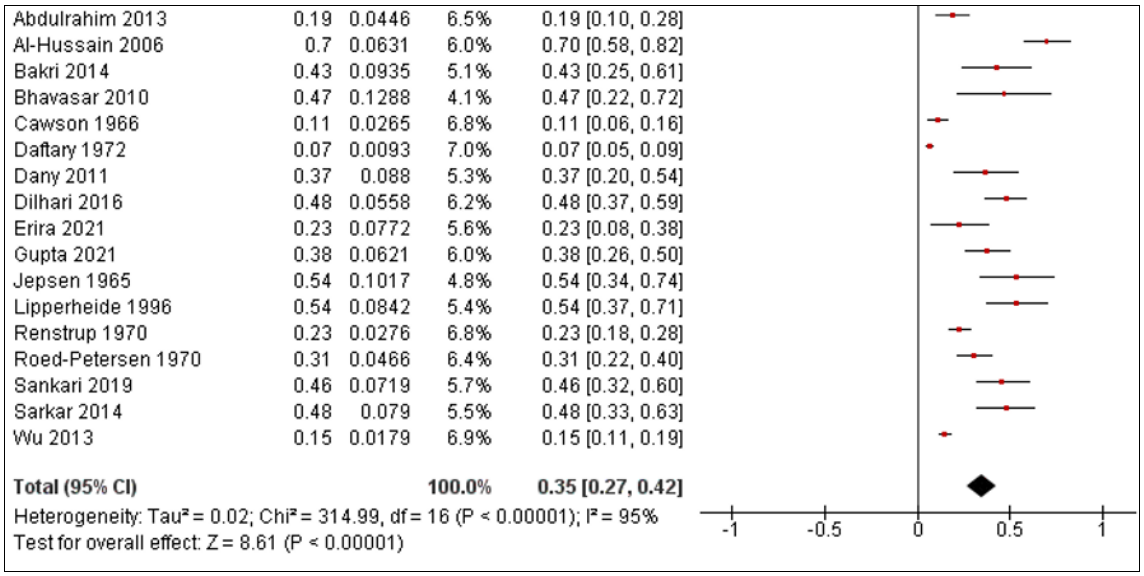

Fig 2: Frequency of *Candida* species detection in oral leukoplakias

The evaluation of other parameters related to *Candida* species detection in oral leukoplakia and the changes in its biological behavior are shown in Table 2.

Three studies [15, 16, 19] analyzed oral leukoplakias infected or not by *Candida* species based on age. Patients with *Candida*-infected oral leukoplakias had a mean age of 3.65 years older than those with non-infected oral leukoplakias. In the statistical analysis, no significant association was found (MD=3.65; 95%CI: -0.69 to 7.99; p=0.10). These same three studies [15, 16, 19] examined the influence of gender in *Candida*-infected leukoplakias, finding a higher frequency of infected oral leukoplakias in males, although without reaching statistical significance (OR=1.22; 95%CI: 0.78 to 1.92; p=0.39).

Four studies [10, 18, 21, 22] focused on the clinical types of oral leukoplakia (non-homogeneous/homogeneous) in *Candida*-infected lesions. *Candida*-infected oral leukoplakias quadrupled their probability of being non-homogeneous types, with highly statistically significant differences (OR=4.07; 95%CI: 2.51 to 6.58. p<0.001).

Five studies [14-16, 18, 21] assessed epithelial dysplasia in patients with oral leukoplakias infected or not by *Candida* species. Patients with Candida-infected oral leukoplakias had a 2.47 times greater risk of being dysplastic lesions, with a statistically significant relationship (OR=2.47; 95%CI: 1.53 to 3.98; p<0.01).

Three studies [14, 15, 21] considered the degree of dysplasia (mild/moderate-severe) in infected or non-infected oral leukoplakias. Although mild dysplasia was more frequent in Candida-infected oral leukoplakias, no statistically significant association was found (OR=2.11; 95% CI: 0.76 to 5.86; p=0.15). In contrast, Candida-infected oral leukoplakias increased by 3.83 times their probability of presenting moderate-severe dysplasia, finding statistically significant differences (OR=3.83; 95%CI: 1.11 to 13.19; p=0.03).

Table 2: Evaluation of different parameters related to *Candida* species detection in oral leukoplakia and the changes in its biological behavior

Parameter	Ref.	Outcome	MD/OR	(95% CI)	I^2	*P*-value
Age (years)	[15,16,19]	CL	MD: 3.65	(-0.69 to 7.99)	56%	0.10
Gender	[15,16,19]	males	OR: 1.22	(0.78 to 1.92)	40%	0.39
Clinical types	[10,18,21,22]	Non-homogeneous	OR: 4.07	(2.51 to 6.48)	0%	<0.001*
Epithelial dysplasia	[14-16,18,21]	CL	OR: 2.47	(1.53 to 3.98)	0%	<0.001*
Mild	[14,15,21]	CL	OR: 2.11	(0.76 to 5.86)	40%	0.15
Moderate-Severe	[14,15,21]	CL	OR: 3.83	(1.11 to 13.19)	0%	0.03*

Ref.: references; **MD:** mean difference; **OR:** Odds Ratio; **(95% CI):** 95% confidence interval; **I^2:** Higgins statistic for heterogeneity (percentage); **CL**: *Candida*-infected oral leukoplakias; *****statistically significant.

4. Discussion

In the present meta-analysis, data from seventeen studies on *Candida* superinfection of oral leukoplakias have been included.

In this study, patients with *Candida*-infected oral leukoplakias had a mean age of 3.65 years older than patients with non-infected leukoplakias, although there was no statistically significant relationship (p=0.10). Of the three studies that analyzed age, two of them [16, 19] agree that patients with *Candida*-infected lesions had a higher mean age, while one [15] did not agree with this result. Advanced age (>60 years old) is a predisposing factor for candidiasis as well as *Candida* superinfection of oral lesions such as oral leukoplakia. This predisposition seems to be related to changes in the oral microbiota and the oral microenvironment [19].

In the present study, *Candida*-infected oral leukoplakia patients were mostly male, although statistical significance was not reached (p=0.39). Of the three studies that evaluated gender, two of them [16, 19] agree that *Candida*-infected leukoplakias were more frequent in men; meanwhile, one [15] found a higher prevalence in women. This apparent greater candidal infection in the male population could be explained by the influence of their harmful habits such as tobacco and/or alcohol consumption. Both one and the other favor fungal proliferation and fungal superinfection of oral lesions, modifying their biological behavior [19].

In this investigation, *Candida*-infected oral leukoplakias quadrupled their probability of being non-homogeneous oral leukoplakias, with highly statistically significant differences (p<0.001). All the studies [10, 18, 21, 22] that considered the clinical types of leukoplakia agreed that most infected oral leukoplakias were non-homogeneous lesions. These non-homogeneous oral leukoplakias are those with the worst biological behavior and, therefore, a greater risk of malignant transformation. The virulence factors of *Candida*, especially nitrosamines and acetylaldehydes -known carcinogenic agents- may induce tissue changes such as epithelial dysplasia in oral leukoplakias. They also promote inflammatory phenomena that are usually accompanied by the appearance of symptoms in these lesions [21].

In the present investigation, *Candida*-infected oral leukoplakias were 2.47 times more likely to be dysplastic lesions, observing a statistically significant association (p<0.01). The five studies [14-16, 18, 21] that focused on this parameter confirmed this relationship between *Candida* superinfection and epithelial dysplasia. In addition, *Candida* superinfection had a significant influence on the probability of moderate-severe dysplasia (p=0.03) but not on mild dysplasia (p=0.15). *Candida*-infected oral leukoplakias increased by 3.83 times the probability of presenting a higher degree of epithelial dysplasia (moderate-severe). All the studies [14, 15, 21] that investigated this parameter corroborated this finding. *Candida* superinfection of oral leukoplakia can cause keratolysis and increased inflammation of the submucosa, thus affecting the epithelial architecture and leading to dysplastic changes and increased risk of malignant degeneration [18]. *Candida* can produce acetylaldehydes that increase the potential for malignant transformation of oral leukoplakias through the induction of severe dysplastic changes. Moreover, *Candida albicans* adhesion, invasion of oral tissues, and damage to oral epithelial cells are influenced by a combination of fungal morphology and activity. *Candida albicans* can invade oral epithelial cells both by induced endocytosis and by active penetration [15]. As the degree of dysplasia increases, the

probability of candidal superinfection of oral leukoplakias increases, being much more frequent in lesions with moderate-severe dysplasia than in those with mild dysplasia. However, it remains to be determined whether *Candida* plays a role in the progression of dysplasia in oral leukoplakias or simply affects the altered tissue [14].

This study has some limitations. The different methods used for *Candida* species detection may have conditioned its prevalence in oral leukoplakias. Neither could different *Candida* species and their possible influence on the biological behavior of the lesions be considered independently. Finally, the high heterogeneity observed in some comparisons demands a cautious interpretation of the results.

5. Conclusions

In this meta-analysis, the estimated prevalence of *Candida*-infected oral leukoplakias was 35%. Non-homogeneous oral leukoplakias quadruple the risk of being infected by *Candida* species ($p<0.001$). Regarding epithelial dysplasia, *Candida*-infected leukoplakias were 2.47 times more likely to be dysplastic lesions ($p<0.001$) and 3.83 times more likely to present moderate-severe dysplasia ($p=0.03$). However, age, gender, or mild epithelial dysplasia were factors with no relevant influence on *Candida* superinfection of oral leukoplakias ($p>0.05$).

6. References

1. Warnakulasuriya S, Johnson NW, Van Der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007; 36(10):575-580.
2. Carrard VC, van der Waal I. A clinical diagnosis of oral leukoplakia; A guide for dentists. Med Oral Patol Oral Cir Bucal. 2018; 23(1):e59-e64.
3. Weerasekera MM, Wijesinghe GK, Sampath A, Dilhari A, Madhumal T, Dilrukshi R, *et al*. The genotypes and virulence attributes of C. albicans isolates from oral leukoplakia. Med Oral Patol Oral Cir Bucal. 2021; 26(6):e786-e794.
4. Aguirre-Urizar JM, Lafuente-Ibáñez de Mendoza I, Warnakulasuriya S. Malignant transformation of oral leukoplakia: Systematic review and meta-analysis of the last 5 years. Oral Dis. 2021; 27(8):1881-1895.
5. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, *et al*. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses [Internet]. Ottawa (Canada): The Ottawa Hospital. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
6. Jepsen A, Winther JE. Mycotic infection in oral leukoplakia. Acta Odontol Scand. 1965; 23:239-256.
7. Cawson RA. Chronic oral candidiasis and leukoplakia. Oral Surg Oral Med Oral Pathol. 1966; 22(5):582-591.
8. Renstrup G. Occurrence of candida in oral leukoplakias. Acta Pathol Microbiol Scand B Microbiol Immunol. 1970; 78(4):421-424.
9. Roed-Petersen B, Renstrup G, Pindborg JJ. Candida in oral leukoplakias. A histologic and exfoliative cytologic study. Scand J Dent Res. 1970; 78(4):323-328.
10. Daftary DK, Mehta FS, Gupta PC, Pindborg JJ. The presence of *Candida* in 723 oral leukoplakias among Indian villagers. Scand J Dent Res. 1972; 80(1):75-79.
11. Lipperheide V, Quindós G, Jiménez Y, Pontón J, Bagán-Sebastián JV, Aguirre JM. *Candida* biotypes in patients with oral leukoplakia and lichen planus. Mycopathologia. 1996; 134(2):75-82.
12. Al-Hussain AAA. The relation of *Candida* growth in the development of "leukoplakia". J Bagh Coll Dentistry. 2006; 18(3):43-47.
13. Bhavasar RS, Goje SK, Takalkar AA, Ganvir SM, Hazarey VK, Gosavi SR. Detection of *Candida* by calcofluor white. Acta Cytol. 2010; 54(5):679-684.
14. Dany A, Kurian K, Shanmugam S. Association of *Candida* in different stages of oral leukoplakia. J Indian Acad Oral Med Radiol. 2011; 23(1):14-16.
15. Abdulrahim MH, McManus BA, Flint SR, Coleman DC. Genotyping *Candida albicans* from *Cándida* leukoplakia and non-*Cándida* leukoplakia shows no enrichment of multilocus sequence typing clades but enrichment of ABC genotype C in *Candida* leukoplakia. PLoS One. 2013; 8(9):e73738.
16. Wu L, Feng J, Shi L, Shen X, Liu W, Zhou Z. Candidal infection in oral leukoplakia: A clinicopathologic study of 396 patients from eastern China. Ann Diagn Pathol. 2013; 17(1):37-40.
17. Bakri MM, Cannon RD, Holmes AR, Rich AM. Detection of *Candida albicans* ADH1 and ADH2 mRNAs in human archival oral biopsy samples. J Oral Pathol Med. 2014; 43(9):704-710.
18. Sarkar R, Rathod GP. Clinicopathologic assessment of *Candida* colonization of oral leukoplakia. Indian J Dermatol Venereol Leprol. 2014; 80(5):413-418.
19. Dilhari A, Weerasekera MM, Siriwardhana A, Maheshika O, Gunasekara C, Karunathilaka S, *et al. Candida* infection in oral leukoplakia: An unperceived public health problem. Acta Odontol Scand. 2016; 74(7):565-569.
20. Sankari SL, Mahalakshmi K. Oral candidal carriage among patients with oral potential malignant disorders: A case-control study. Pesqui Bras Odontopediatria Clin Integr. 2019; 19:e4802.
21. Erira AT, Navarro AFR, Robayo DAG. Human papillomavirus, Epstein-Barr virus, and *Candida albicans* co-infection in oral leukoplakia with different degrees of dysplasia. Clin Exp Dent Res. 2021; 7(5):914-923.
22. Gupta SR, Xess I, Singh G, Sharma A, Gupta N, Mani K, *et al.* Therapeutic implications of *Cándida* phenotypes, virulence factors and antifungal sensitivity in Oral leukoplakia. J Oral Biol Craniofac Res. 2021; 11(2):354-360.