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Genetic Mechanisms of Oral Leukoplakia: A Systematic Review

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ABSTRACT

Objectives: Oral leukoplakia (OL) is the most common type of oral potentially malignant disorder. It is currently managed through lesion removal either by laser excision or resection. However, its multifactorial aetiology often results in recurrence and cancer transformation. Therefore, findings on novel biomarkers are emerging to understand OL formation and progression towards malignancy. We performed a systematic review to identify the genetic factors for the risk of OL conditions. **Methods:** The study protocol was registered in PROSPERO, ID: CRD42024497161. We searched PubMed, MEDLINE, Scopus, ScienceDirect, Web of Science, ClinicalKey and Wiley databases from 2018 to 2023. The study was conducted following PRISMA guidelines and articles were selected based on predefined inclusion criteria. Genomic profiles of OL tissues collected from study patients were summarised based on outcome determinants of predictive or diagnostic markers, in relation to OL histopathological features. Results: Seventeen studies met the inclusion criteria. **Results** showed that OL formation and progression involve genetic factors such as cytokines, proliferation antigen, major histocompatibility complex and CD molecules of lymphocytes, caspase, immune checkpoint, oral cancer key genes, nuclear proteins and transcription factors, toll-like receptor, macrophage and polycomb complex. Among them, the transcription factor p53 is the most investigated factor. However, the cytokine was found to play critical roles in OL progression towards the advanced stages, and is closely associated with dysplastic changes. **Conclusion:** Exposure to genetic alterations results in OL malignant transformation. Future studies on differential cytokines profiling of differentiated dysplasia, may reveal the novel stage-wise biomarkers for molecular pathological grading.

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INTRODUCTION

Oral leukoplakia (OL) is the most common type of oral potentially malignant disorders (Mello et al., 2018). Unfortunately, it is uncharacterised clinically and has no specific histological feature. Leukoplakia is defined as a predominantly white patch or plaque that cannot be characterised clinically or pathologically as any other disorder (Kramer et al., 1978); OL carries an increased risk of cancer development either in or close to the area of the leukoplakia or elsewhere in the oral cavity or the head-and-neck region (Warnakulasuriya et al., 2007).

OL lesions may occur on the buccal mucosa, tongue, gingiva and lips (Abidullah et al., 2014). Histologically, the buccal mucosa is lined by non-keratinised stratified squamous epithelium, while the dorsal tongue is lined by a keratinised epithelium. Normal cells of the oral mucosa may become cancer cells through a progression from normal to hyperplasia, with or without dysplasia. Histopathologically, it may show epithelial atrophy (thinned) or hyperplasia (thickened) and may, or may not demonstrate dysplasia (Li et al., 2021). There are several criteria for dysplasia that refers to the features of architectural changes of the epithelium. Most of the oral premalignant lesions (OPLs) show basal cell hyperplasia, loss of epithelium stratification and hyperkeratosis (Ranganathan & Kavitha, 2019). Apart from having these variable behavioural patterns, OL has an assessable tendency to malignant transformation (Warnakulasuriya et al., 2007).

Clinical features of OL can range from a thin, greyish surface that demonstrates a white translucent quality with ill-defined margins, and generally smooth surface qualities to discrete, sharply marginated, thick and opaque plaques (Sciubba, 2021). The key etiological element for these conditions is still unclear. During evaluation of its clinical features, OL can be classified into two types: homogenous leukoplakia and non-homogenous leukoplakia (Carrad & van der Waal, 2017). While homogenous leukoplakia presents as well-demarcated, uniform white plaque that is often fissured, its non-homogeneous counterpart takes several forms (Jessri et al., 2019). Those forms are verrucous leukoplakia, nodular leukoplakia and erythroleukoplakia (Jessri et al., 2019). Clinically, OL is managed by a discussion with the pathologist and removal of the lesion either by laser excision or resection (Lodi & Porter, 2008; Kuribayashi et al., 2012). Further, the clinician re-evaluates for recurrence or the development of new leukoplakia, and patients with leukoplakia exhibiting carcinoma in situ and invasive oral squamous cell carcinoma (OSCC) should be referred to a cancer centre (Jessri et al., 2019). However, current limitations such as unavailability of objective predictive and diagnostic markers of leukoplakia lesions, result in limited preventative measures, failure of early detection and accurate diagnosis, and treatment options.

The development and progression of OL lesions are believed to have resulted from genomic instability. With regards to the genetic/chromosomal alterations influencing the formation and progression of OL, during the evolution of OPLs progression, for example, it has been shown to be strongly infiltrated with CD8+ T cells (Rangel et al., 2022). In early grade dysplasia, there is an increased immunosuppressive M1 TAM gene, followed by M2 TAM, Treg and MDSCs genes in the middle grade, and eventually PD-1, PD-L1, and A2AR genes in the late stage (Rangel et al., 2022).

In clinical research of OL, new findings are emerging on the genetic factor in the pathogenesis of leukoplakia. Researchers are investigating novel biomarkers that may aid in the understanding of leukoplakia formation and progression towards its malignant transformation. A study of integrative genome-wide analysis, for example, showed significant amplification of 8q24.3, deletion of 8p23.2, and dysregulation of DERL3, EIF5A2, ECT2, HOXC9, HOXC13, MAL, MFAP5 and NELL2 genes in leukoplakia tissues, which was further corroborated with clinical manifestations of advanced gingivobuccal cancer (Bhosale et al., 2017).

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The clinical studies of OL are done either through i. prospective studies that analyse the progression of OL with long-term follow-up outcomes, ii. retrospective studies which compare between the groups with and without OL, or iii. by single case studies. To date, there has been strong evidence of potential predictive markers reported by the prospective studies and possible diagnostic markers reported by the retrospective studies. These findings may bring insights into a better understanding of OL etiopathogenesis. Therefore, this systematic review aims to: (i) identify the genetic factors influencing the formation and progression of OL and (ii) investigate the relationship between the biomarkers and the histopathological features of OL, with regards to the risk for malignant transformation.

MATERIALS AND METHODS

The study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO), ID: CRD42024497161.

Search strategy

The articles were systematically searched in accordance with the PRISMA 2020 guidelines. It was conducted in three stages involving article i. identification, ii. screening, and iii. included, in parallel with the predefined inclusion criteria (Table 1). In identification stage, articles were searched through PubMed, MEDLINE, Scopus, ScienceDirect, Web of Science, ClinicalKey and Wiley databases, from the year 2018 to 2023, based on Booleans ‘genetic’ AND ‘oral dysplastic lesions’ AND ‘oral leukoplakia’. The articles obtained from all databases were downloaded and transferred to the reference management software EndNote to ease the tracking of articles, in terms of the number of articles identified and the number of duplicates removed, as well as for the purpose of sharing the access of EndNote library between the reviewers at the end of article screening.

Procedure for study selection

Two independent reviewers were involved in the identification phase (article search through databases), and the same independent reviewers were involved in the screening phase (article with title, abstract and full text available, that comprises predefined inclusion criteria). An addition of one more independent reviewer (3 reviewers) were involved in the included phase (article that concludes the predictive or diagnostic markers of oral leukoplakia). Discrepancies were resolved by an addition of one more independent reviewer (4 reviewers) through inter-examiner calibration. The search strategy was based on Boolean ‘genetic’ AND ‘oral dysplastic lesions’ AND ‘oral leukoplakia’. The selection criteria include (i) Type of study, (ii) Type of report, (iii) Publication date and (iv) Language. The inclusion and exclusion criteria used to select studies is shown in Table 1.

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Table 1. Inclusion and exclusion criteria.

	Inclusion criteria	Exclusion criteria
i. Type of study	a. Prospective studies that analyse progression of oral leukoplakia with long-term follow-up outcomes, or b. Retrospective studies comparing the groups with and without oral leukoplakia.	Single case studies.
ii. Type of report	Studies that report on: a. Predictive markers (for Prospective studies), or b. Diagnostic markers (for Retrospective studies).	Studies that do not report on signalling pathways/molecular markers.
iii. Publication Date	Articles between 2018 - 2023.	Articles before 2018, and articles published after 2023.
iv. Language	Articles in English language.	Articles in other languages.

Strategy for data synthesis

1. **Details of demographic characteristic of the study patients**

The details of demographic characteristics of the study patients involving age, gender, country, cigarette smoking, alcohol consumption, areca (betel) nut use or mixed habit were recorded.

2. **Determination of outcome determinant and histological features**

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The outcome parameters investigated in the selected studies were the genetic factors of signalling pathways as hallmarks of OL. The outcome determinants include upregulation or downregulation of the predictive or diagnostic markers, in relation with the histopathological features. The outcomes were summarised in a table, based on authors and their findings.

3. **Genetic factors, and the risk for oral leukoplakia conditions**

The groups of the predictive and diagnostic markers and the risk for OL conditions obtained from patients of each study were described. The biomarkers were further summarised in a table, with regards to their relation to the histological features of normal, hyperplasia, dysplasia or transformed to OSCC.

4. **Relationship between biomarker and histopathology of oral leukoplakia transformation towards malignancy**

The biomarkers involved in the normal group, and groups at risk for OL conditions including hyperplasia, dysplasia and cancer were further summarised and drawn in a diagram to show the relationship between the biomarkers and their histopathology related to leukoplakia transformation towards malignancy.

RESULTS

Articles selection

499 articles were obtained during the Identification stage following searching through the databases. At the end of Screening stage, the duplicates were removed, and research articles in English language with full text available, which reported the findings on prospective and/or retrospective studies in human were chosen, where 68 articles were obtained. During the Included stage, only research articles which concluded on the predictive markers (for prospective studies), or diagnostic markers (for retrospective studies) were chosen, where 40 articles were obtained. At the end of the third stage, inter-examiner calibration was done to finalise selected articles for the present review, where 17 research articles were finally selected for the present systematic review at the end of the included stage (Figure 1).

Details of demographic characteristic of the study patients of oral leukoplakia

The details of demographic characteristics of the study patients of OL include the age, gender, country and hospital/ institute of sample origin, habit, and year of sample collection. More than half of study patients were in the age range of 40 to 50 years old. Both genders were involved in the majority of the studies. The habit of tobacco/ cigarette smoking, and alcohol consumption were reported in nearly half of the studies. The details of demographic characteristics in the seventeen studies are summarised in Table 2.

Summary of biomarkers of oral leukoplakia and their histological features

The biomarkers of all the seventeen studies were described based on the genetic factors, in relation to their histopathological features. The presence of dysplasia and/or without dysplasia were investigated by three-fourth of the studies. The biomarkers of OL with and/ or without dysplastic changes are summarized in Table 3.

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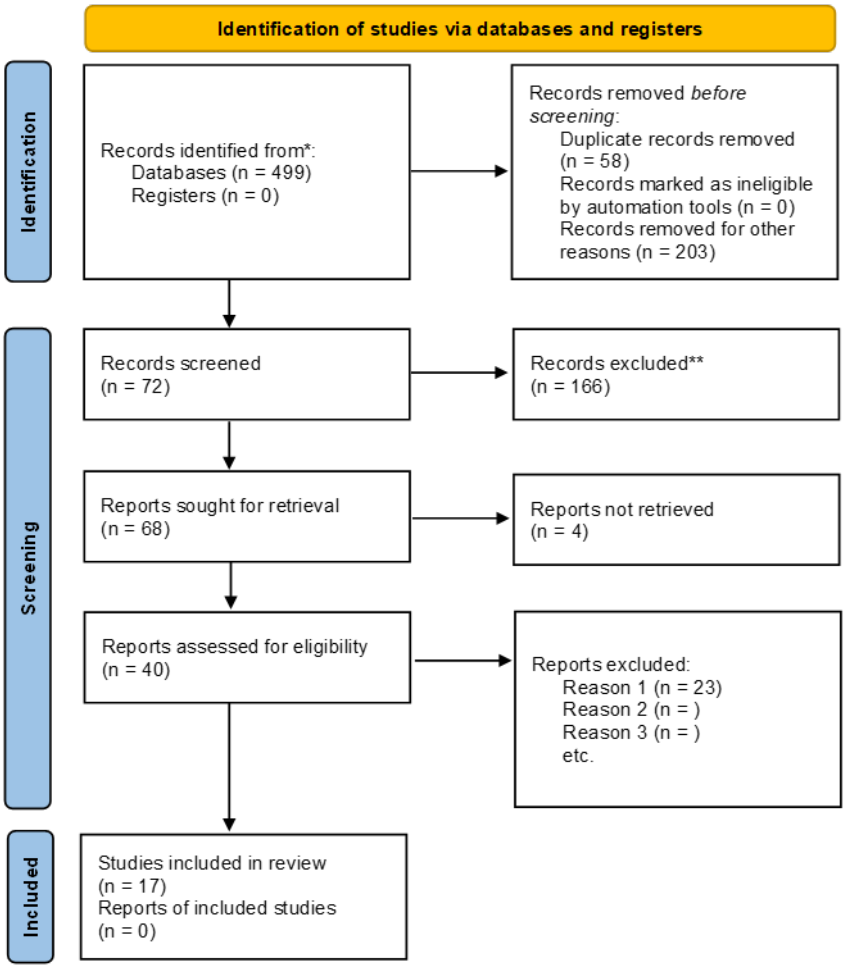

Fig. 1. PRISMA 2020 flow diagram

The search and selection of the articles were carried out in three stages. In the first stage (Identification), the articles were searched based on Booleans, through databases. In the second stage (Screening), the duplicates were removed, and research articles in English language with full text available, which reported the findings on prospective and/or retrospective studies in human were chosen. In the third stage (Included), only research articles which concluded on the predictive markers (for prospective studies), or diagnostic markers (for retrospective studies) were chosen. At the end of the third stage, inter-examiner calibration was done to finalize selected articles for the present review.

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Table 2. Summary of the details of demographic characteristics of the study patients of oral leukoplakia.

No.	References	Age (year old)	Gender	Country	Sample obtained from	Habit	Sample taken (year)
1.	Babiuch et al. (2020)	N/A	N/A	Krakow, Poland	Jagiellonian University Medical College.	N/A	2011 - 2015
2.	Beevi et al. (2019)	N/A	N/A	Kerala, India	Educare Institute of Dental Sciences.	N/A	N/A
3.	Dafar et al. (2022)	51 - 57	Female	Gothenburg, Sweden	Clinic of Oral Medicine, Public Dental Service, Gothenburg, Sweden.	Tobacco	N/A
4.	Dikova et al. (2021)	67 - 68	Male & female	Valencia, Spain	University General Hospital of Valencia.	N/A	2017 - 2020
5.	Gan et al. (2022)	N/A	N/A	Malaysia	National Institute of Health Malaysia.	N/A	N/A
6.	Ghosh et al. (2022)	32 - 70	Male & female	Kalyani, India	National Institute of Biomedical Genomics, Kalyani.	Tobacco , alcohol	N/A
7.	Ries et al. (2021)	23 - 92	Male & female	Germany	University Hospital Erlangen and University Hospital Halle (Saale).	N/A	1997 - 2015
8.	Jawert et.al (2022)	39 - 93	Male & female	Sweden	Public Dental Health Service, Gothenburg, Sweden.	N/A	N/A

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9.	Klein et al. (2020)	N/A	Male & female	Brazil	Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul.	Tobacco, alcohol	2000 - 2014
10.	Li et al. (2023)	33 - 58	Male	India, China, USA, Argentina, Iran, Thailand	N/A	Tobacco, alcohol	2023
11.	Sharma et al. (2019)	N/A	Male & female	India	Rajiv Gandhi Research Centre and Hospital, Noida, India.	Tobacco, alcohol	N/A
12.	Singla et al. (2018)	31 - 40	Male & female	India	Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.	Tobacco, alcohol	N/A
13.	Sundberg et al. (2021)	35 - 81	Male & female	Sweden	ORA-LEU-CAN database, Sweden.	Tobacco, alcohol	2011 - 2018
14.	Weber et al. (2020)	23 - 92	Male & female	Germany	University Hospital Erlangen and University Hospital Halle (Saale).	N/A	1994 - 2014
15.	Xu et al. (2021)	49 - 53	Male & female	China	Shanghai Ninth People's Hospital.	N/A	N/A

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16.	Yao et al. (2023)	N/A	Male & female	China	NCBI Gene Expression Omnibus (GEO) database.	N/A	N/A
17.	Yoshida et al. (2019)	28 - 95	Male & female	Japan	Aichi-Gakuen University School of Dentistry, Okazaki City Hospital, Aichi Saiseikai Hospital, Yokkaichi Municipal Hospital.	Tobacco , alcohol	1991 - 2015

N/A : Information not available

Table 3. Summary of Biomarkers of Oral Leukoplakia.

17 studies assessed genetic factors	1. Babiuch et al. (2020), high expression of IL-8 in OL tissues without dysplasia, and high expression of TNF-α in OL with dysplasia, and OSCCs. 2. Beevi et al. (2019), high expression of Ki-67 in OL tissues with dysplasia. 3. Dafar et al. (2022), expression of Ki-67 in OL tissues without dysplasia. Expression of CD1a, CD3 and CD20 in OL without dysplasia. 4. Dikova et al. (2021), OL progression towards OSCC involves IL-6, IL-8, TNF-α, HCC-1 and PF-4, while IL-6 and TNF-α may indicate OSCC progression in the early towards the advanced stages.

	5. Gan et al. (2022), dysregulation of CTNNB1, PTEN and JAK2 in high-risk OL with dysplasia. Moderate and severe dysplasia of the non-immune reactive subtype are likely to progress to OSCC. 6. Ghosh et al. (2022), mutation of the CASP8 gene in OL with dysplasia. Mutations in the other key genes including TP53, NOTCH 1 and HRAS. 7. Ries et al. (2021), expression of PD-1 and PD-L1 in epithelial layers of OL tissues without malignanttransformation, as well as OL tissues that had undergone malignant transformation within 5 years.overexpression of PD-1 and PD-L1 protein in epithelial layers of OL tissues that had undergone malignant transformation within 5 years. 8. Jäwert et al. (2022), amplification/gain in the EGFR and CCND1 genes, and loss of CDKN2A gene in OL with and without dysplasia. alteration of EGFR and loss of CDKN2A gene in OL (dysplastic and non-dysplastic) progressing to OSCC. 9. Klein et al. (2020), highest expression of Ki-67 in OSCC samples, and high expression of Ki-67 in OL tissues with and without dysplasia. Expression of BMI-1 in non-dysplastic and dysplastic OL, and OSCC. 10. Li et al. (2023), no association between the cases of TP53 codon 72 polymorphisms with both the onset or progression of OL into OSCC. 11. Sharma et al. (2019), association between genetic polymorphism of TLR 9 and TLR 4 with 14-fold higher risk for OL as compared to control.

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	12. Singla et al. (2018), overexpression of p53 protein associates with progression risk of OL with mild dysplasia and OSCC. High expression of EGFR in OL with mild dysplasia and OSCC. 13. Sundberg et al. (2021), association of p53 and p63 with high recurrence risk of OL with and without dysplasia. 14. Weber et al. (2020), increase of M2 polarization in dysplastic OL with malignant transformation within 5 years. 15. Xu et al. (2021), expression of hsa_circ_0060927 in normal, OL with dysplasia and OSCC samples, with highest expression in OSCC. 16. Yao et al. (2023), involvement of FN1, STAT1, COL2A1, COL10A1 and COL4A6 in transformation of OL towards early stage OSCC. 17. Yoshida et al. (2019), high expression of p62 in the nucleus of epithelium of OL tissues with dysplasia.

Genetic factors, and the risk for oral leukoplakia conditions

The genetic mechanisms involved in the formation and progression of OL were cytokines, proliferation antigen, major histocompatibility complex and CD molecules of lymphocytes, caspase, immune checkpoint, oral cancer key genes, polycomb complex, nuclear proteins and transcription factors, toll-like receptor, macrophage, circular RNAs and differentially expressed genes (DEGs). The findings of the seventeen studies were described.

1. **Cytokines**

Cytokines are small proteins that transmit intercellular signal in cell replication, differentiation, survival, and transformation. They are regulated by the transcription factor, nuclear factor-kappa B (NF-κB) pathway. The four proinflammatory NF-κB dependent cytokines including interleukins IL-1α, IL-6, IL-8 and tumour necrosis factor-alpha (TNF-α) are expressed in tissue specimens and saliva of patients with OL and OSCC (Babiuch et al., 2020). Among these four, IL-8 could play a leading role in the malignant transformation process within the oral mucosa. IL-8 was not present in normal oral mucosa samples, however, was present in OL samples without dysplasia, and highly increased within all layers of

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epithelium of OSCCs. TNF-α on the other hand, is markedly increased in OL samples with dysplasia and OSCCs, when compared with the normal group. Salivary concentrations of IL-6, IL-8 and TNF-α are increased in patients with OSCCs than in patients with OL without dysplasia.

Another study reported that IL-6, IL-8, TNF-α, CC chemokine (HCC-1) and platelet factor-4 (PF-4) may be strongly involved in the OL progression towards OSCC, while IL-6 and TNF-α may indicate OSCC progression in the early towards the advanced stages (Dikova et al., 2021). The four proinflammatory cytokines including interleukins IL-6, IL-8 and TNF-α, and chemotactic cytokines monocyte chemoattractant protein-1 (MCP-1) and HCC-1 are highly present in the saliva of patients with both the homogenous and proliferative OL when compared to the normal tissues, and no difference was detected between the two groups of clinical forms of OL.

2. Marker of cell proliferation

Ki-67 is a large protein, involved during the interphase and mitosis, thus regulating cell cycle progression. It is highly expressed in cells undergoing cell cycle and being downregulated in the resting G0 cells, and therefore has been used as a marker of cell proliferation for human tumour cells. Ki-67 could be an applicable marker of cell proliferation correlating well with OL progression. The Ki-67 proteins are expressed in both oral lichen planus and OL without dysplasia (Dafar et al., 2022). The mean Ki-67 positive cell count is highly increased in OL tissue with dysplasia in comparison to normal mucosa (Beevi et al., 2019). The Ki-67 proteins was detected highest in OSCC samples and are highly expressed in OL tissues (dysplastic and non-dysplastic) and tissues with inflammatory hyperplasia, in comparison to the normal group (Klein et al., 2020). However, no difference was reported when compared between the two OL groups.

3. Major histocompatibility complex and cd molecules of lymphocytes

The major histocompatibility complex (MHC) and clusters of differentiation (CD) are surface molecules that help the immune system recognize foreign substances and play a role in intercellular communication. The MHC molecules bind to peptide fragments of pathogens and display them on the cell surface for recognition by the appropriate T cells. A distinct immune response presents in high-risk OL with dysplasia (Gan et al., 2022). OL with moderate and severe dysplasia have three distinct subtypes; the immune cytotoxic, non-cytotoxic and non-immune reactive. The non-immune reactive subtype showed upregulation of genes involved in the stromal microenvironment and cell cycle. The lack of T cell infiltration and activation in the non-immune reactive subtype is due to the dysregulation of CTNNB1, PTEN and JAK2. Moderate and severe dysplasia of the non-immune reactive subtype that demonstrated high lymphocyte infiltration and upregulation of genes involved in immune surveillance (MHC, T cell, B cell, cytolytic activity) and immune suppression (immune checkpoints, T regulatory cells, tumor associated macrophages) are likely to progress to OSCC.

Another study reported that CD molecules are highly expressed on the inflammatory cells, and therefore is widely used as a marker of identification of leukocytes and lymphocyte subset. The three inflammatory cells including Langerhans cell (CD1a), T cell (CD3) and B cell (CD20) are expressed in tissue specimens of both patients with oral lichen planus and OL without dysplasia (Dafar et al., 2022). Among these three, CD20 could play a sturdy role in immune activation of OPLs such as oral lichen planus. Chronic inflammation profiles of OL without dysplasia, however, reported on lower expression of CD3 and CD20 when compared to the oral lichen planus samples, while no difference for CD1a expression between the two groups.

4. Caspase

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Caspases are proteases that play role in cell regulatory networks controlling inflammation and cell death. Signalling pathway initiated by signals such as TNF-α lead to extrinsic apoptosis through activation of Caspase-8 (CASP8), followed by caspase-3 (CASP3) cascade. Studies have indicated that TNF-α induces apoptosis through caspase-8 activation, while caspase-8 inhibition leads to induction of necroptosis. Mutation in the CASP8 gene was identified in OL with dysplasia (Ghosh et al., 2022). Additional to CASP8 mutation, mutations in the other key genes including TP53, NOTCH 1 and HRAS were also identified in gingivobuccal oral cancer tissue. The progression to malignancy is associated with immune suppression through infiltration of regulatory T-cells, depletion of cytotoxic T-cells, antigen-presenting dendritic cells and increase of inflammation.

5. Immune checkpoints

Immune checkpoints are regulators of the immune system, expressed on the immune cells including T-cells, regulatory B- and T-cells, dendritic cells, natural killer cells and M2-type macrophages. The programmed cell death receptor 1 (PD-1) protein and programmed cell death ligand 1 (PD-L1) are expressed in epithelial layers of OL tissues without malignant transformation as well as OL tissues that had undergone malignant transformation within 5 years (Ries et al., 2021). Among these two, PD-L1 may establish immunosuppressive microenvironments which eventually contribute to malignant transformation. PD-1 protein is highly expressed in both epithelial and subepithelial compartments of OL tissues that had undergone malignant transformation within 5 years, as well as the normal tissue, in comparison to the OL tissues without malignant transformation, and OSCC. The expression of PD-1 protein is increased by 5-fold in only the epithelial compartment of OL tissues that had undergone malignant transformation within 5 years compared to the OL tissues without malignant transformation. The expression of PD-L1 protein on the other hand, is found highest in the OCSS tissues, is 10-fold higher in the OL tissues that had undergone malignant transformation within 5 years compared to the OL tissues without malignant transformation, and the lowest expression was detected in the normal tissue.

6. OSCC key genes

Epidermal growth factor receptor (EGFR) is a protein on the cells that helps the cells grow, while Cyclin D1 (CCND1) gene is a protein coding gene which regulates cell cycle and involved in tumour growth and progression. Cyclin-dependent kinase inhibitor 2A (CDKN2A) gene on the other hand, is a regulator of cell division that play role as tumour suppressor. EGFR gene is highly expressed in OL with mild dysplasia and OSCC tissues when compared to normal group (Singla et al., 2018). Another study reported that amplification/gain in the EGFR and CCND1 genes, and loss of CDKN2A gene were identified in OL with and without dysplasia (Jäwert et al., 2022). Among these three, loss of CDKN2A gene was found more common in OL tissues compared to the OSCC, while copy number alterations (amplification/gain) of EGFR and CCND1 genes are more common in OCSS in comparison to OL.

7. Polycomb complex protein BMI-1

B-cell-specific Moloney murine leukaemia virus integration region 1 (BMI-1) is a member of Polycomb complex protein which regulates cell proliferation and differentiation. BMI-1 is expressed in tissue specimens of both dysplastic and non-dysplastic OL patients and OSCC (Klein et al., 2020). Its expression may be associated with the occurrence of dysplastic changes, and high-grade epithelial dysplasia was found to be associated with malignant transformation. The expression of BMI-1 is found lowest in the normal tissue, and increasing positivity were observed in tissues with inflammatory hyperplasia, followed by non-dysplastic OL and dysplastic OL, and the highest expression was detected in the OCSS tissue.

8. Nuclear proteins and transcription factors

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To date, overexpression of p53 protein has been reported to be significantly associated with progression risk of OL with mild dysplasia and OSCC when compared to normal group (Singla et al., 2018). Another study reported that the expression of p53 and its homolog p63 proteins are associated with higher recurrence risk of dysplastic and non-dysplastic OL (Sundberg et al., 2021). The TP53 gene provides instruction for the making of tumour protein p53, which accumulates in the nucleus and play role as a transcription factor. TP53 gene regulates cell proliferation by keeping the cells from dividing in an uncontrolled way, and thus involved in tumour suppression. Investigation on the TP53 genetic typing however, revealed that TP53 genetic typing could not influence p53 protein expression in OL. No association was found between the cases of TP53 codon 72 polymorphisms with both the onset or progression of OL into OSCC for all genetic models including allele C vs G, homozygote CC vs GG, heterozygote GC vs GG, dominant GC+CC vs GG, and recessive CC vs GG + GC (Li et al., 2023).

The p62 proteins are present in both the nucleus and cytoplasm, which facilitates protein degradation through autophagy, involved in controlling cancer development. p62 protein is aggregated and highly expressed in the nucleus of epithelium of OL tissues with dysplasia (Yoshida et al., 2019). The expression of p62 in the cytoplasm however, was not associated with epithelial dysplasia of OL tissues. Further investigation on the relationship between p62 parameters including p62 expression in the nucleus and p62 aggregation with other biomarkers including Ki-67 and p53, revealed a significant association between p62 in the nucleus and p53.

9. **Toll-like receptors (TLRs)**

Toll-like receptors (TLRs) are cell surface pattern recognition receptors (PRRs) which function in innate immune response, and are able to initiate adaptive immune response. Genetic polymorphism of TLR 9 and TLR 4 are associated with 4-fold and 14-fold higher risk for OL respectively (Sharma et al., 2019). The frequency of variant allele ‘C’ of TLR 9, variant allele ‘T’ of TLR 6, and variant allele ‘G’ of TLR 4 are associated with an increased risk for OSCC. In addition, human papillomavirus (HPV) was detected more frequently in OL samples of patients when compared to the normal group.

10. **Macrophages**

Macrophages are innate immune cells which are able to initiate adaptive immune responses. The progression of OL to OSCC involves complex inflammatory processes, where highest macrophage infiltration and strongest M2 polarization were detected in OSCC samples. Macrophage infiltration and M2 polarized macrophages are increased in dysplastic OL with malignant transformation within 5 years, when compared to OL without malignant transformation (Weber et al., 2020). A significant shift of macrophage infiltration was observed towards the epithelial compartment of the OL tissue with malignant transformation.

11. **Circular RNA**

Circular RNA (circRNA) is single stranded RNA which regulates gene expression, and play role in the process of miRNA sponges. The Hsa_circ_0060927 is expressed in tissue specimens of normal, OL with dysplasia and OSCC with highest expression observed in the OSCC group (Xu et al., 2021). To date, circRNA has been reported to act as a molecular sponge to relieve inhibition of miRNA on mRNA. Investigation on a few of the downstream miRNAs matches to hsa_circ_0060927 including miR-195-5p, miR-15b-5p and miR-93-3p revealed the strongest binding between hsa_circ_0060927 and miR-195-5p. Further investigation revealed TRIM14 as the downstream target gene of miR-195-5p. Hsa_circ_0060927 may play a critical role in the malignant transformation of OL to OSCC through the hsa_circ_0060927/ miR-195-5p/ TRIM14 axis.

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12. **Differentially expressed genes (DEGs)**

Differentially expressed genes (DEGs) are genes that show significant differences in expression levels between two or more groups, such as a disease group and a healthy control group. Profiling data from the gene expression analysis of OL and early stage OSCC (GEO accession GSE85195) screening revealed that fibronectin 1 FN1, signal transducer and activator of transcription 1 STAT1, collagen type II α1 COL2A1 chain, collagen type X α1 COL10A1 chain and collagen type IV α6 COL4A6 chain may serve as independent biomarkers for malignant transformation of OL towards OSCC (Yao et al., 2023).

Relationship between biomarkers and histopathology of oral leukoplakia conditions towards malignant transformation.

The relationship between the biomarkers and their histopathological features of OL, with regards to the risk for malignant transformation were summarised in Table 4 and Figure 2. In genetic involvement, the cytokines show a close relation to the dysplastic changes.

Table 4. Genetic factors, and the risk for oral leukoplakia conditions.

Genetic						**Author**
Biomarkers		**Histological feature**				
		Normal	**Hyperplasia**	**Dysplasia**	**OSCC**	
Cytokines	IL-1α		+	+	+	Babiuch et al. (2020)
	IL-6	↓			↑↑	
			↑	↑	↑↑	Dikova et al. (2021)
	IL-8	↑	↑	↑	↑↑	Babiuch et al. (2020)
			↑	↑		Dikova et al. (2021)
	TNF-α	↓			↑↑	Babiuch et al. (2020)
			+	↑↑	↑↑	

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	HCC-1		↑	↑		Dikova et al. (2021)
	PF-4		↑			
	MCP-1		↑	↑		
Marker of cell proliferation	Ki-67			↑↑		Beevi et al. (2019)
			↑			Dafar et al. (2022)
		↑	↑↑	↑↑	↑↑	Klein et al. (2020)
		+		↑	↑	Sundberg et al. (2021)
Major histocompatibility complex and CD molecules of lymphocytes	CTNNB1			↑		Gan et al. (2022)
	PTEN			↑		
	JAK2			↑		
	CD1a	+	↑			Dafar et al. (2022)
	CD3	+	↑	↑	↑	
	CD20		↑			
Caspase	CASP8			↑		Ghosh et al. (2022)
	PD-1		↑	↑↑	↑↑	Ries et al. (2021)

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Immune checkpoints	PD-L1		↑	↑↑	↑↑	
OSCC key genes	EGFR	+	↑	↑↑	↑↑	Singla et al. (2018)
	CDKN2A		↓			Jäwert et al. (2022)
Polycomb BMI-1	BMI-1		↑	↑	↑	Klein et al. (2020)
Nuclear proteins and Transcription factors	p53	+	↑	↑↑	↑↑	Singla et al. (2018)
		+	↑	↑	↑	Sundberg et al. (2021)
	p63	+		↑		
	TP53			-		Li et al. (2023)
	p62	+		↑		Yoshida et al. (2019)
Tol-like receptors (TLRs)	TLR9	↓	↑	↑	↑	Sharma et al. (2019)
	TLR4	↓	↑	↑	↑	
Macrophages	M2 polarization			↑	↑	Weber et al. (2020)

Cicular RNA	Hsa_circ_0060927		↑	↑	↑	Xu et al. (2021)
Differentially expressed genes (DEGs)	FN1	+		↑	↑	Yao et al. (2023)

+ = expressed

↑ = increased

↑↑ = highly increased

↓ = decreased

↓↓ = highly decreased

- = no association

IL-1α: interleukin-1α, IL-6: interleukin-6, IL-8: interleukin-8, TNF-α: tumour necrosis factor-alpha, HCC-1: CC chemokine, PF-4: platelet factor-4, MCP-1: monocyte chemoattractant protein-1, Ki-67: Kiel original clone 67, CTNNB1: catenin beta 1, PTEN: phosphatase and tensin homolog deleted on chromosome ten, JAK2: Janus Kinase 2, CD1a: cluster of differentiation 1a, CD3: cluster of differentiation 3, CD20: cluster of differentiation 20, CASP8: caspase-8, PD-1: programmed cell death receptor 1, PD-L1: programmed cell death ligand 1, EGFR: Epidermal growth factor receptor, CDKN2A: Cyclin-dependent kinase inhibitor 2A, BMI-1: B-cell-specific Moloney murine leukemia virus integration region 1, p53: tumour protein 53, p63: tumour protein 63, TP53: gene for tumour protein 53, p62: ubiquitin-binding protein p62, TLR9: toll-like receptor 9, TLR4: toll-like receptor 4, M2: macrophage M2 subcategory, Hsa_circ_0060927: circular RNA Hsa_circ_0060927, FN1: fibronectin 1, STAT1: signal transducer and activator of transcription 1, COL2A1: collagen type II α1 chain, COL4A6: collagen type IV α6, RARβ2: retinoic acid receptor-beta 2, p16INK4a: INK4 family member p16, FAT1: FAT atypical cadherin 1, GLDC: glycine decarboxylase, HOXB13: Homeobox B13, EZH2: enhancer of zeste homolog 2, miR-372: microRNA-372, miR-10b: microRNA-10b, circHLA-C: circular RNA HLA-C, miR-375: microRNA-375

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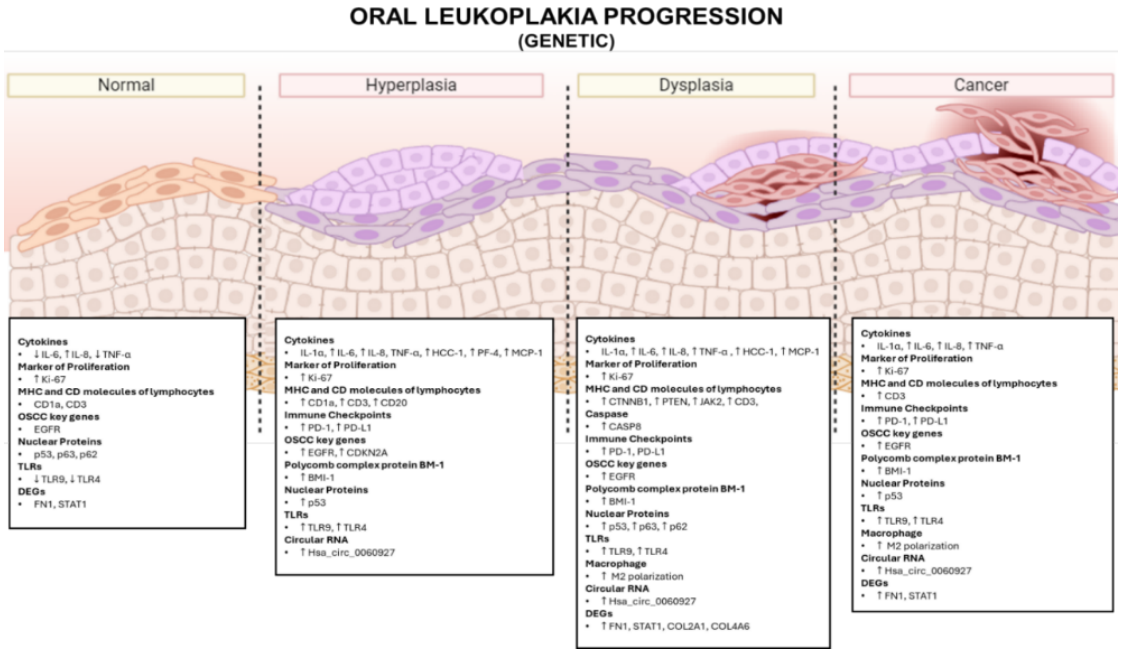

Fig. 2. Genetic factors and the risk for oral leukoplakia conditions.

The genetic factors of seventeen OL studies were summarised based on the histopathological features involving the normal, hyperplasia/ without dysplasia, dysplasia and OSCC. The risk for OL transformation towards OSCC involves the histologic and molecular biomarkers.

DISCUSSION

In the current clinical studies, the factors related to OL occurrence and progression into OSCC are generally genetic, epigenetic, microbial, habitual and lifestyles (Kumari et al., 2022). Evaluation of the chromosomal aberrations and determination of pattern of genes expressed at the level of genetic transcription under specific circumstances are considered as the main parameter for genetic factor.

The clinical studies of OL are done either through i. prospective studies that analyse the progression of OL with long-term follow-up outcomes, and ii. retrospective studies which compare between the groups with and without OL. The prospective studies may be able to integrate the pre-diagnostic and diagnostic markers for an early detection of malignant transformation (Pesch et al., 2014). The retrospective studies on the other hand, which reported on the feature of determinants including advanced age, sex, OL size, non-homogenous type of OL, hyperplasia, dysplasia and the higher grades of dysplasia may provide a clearer picture on the association between these risk factors with its malignant transformation (Warnakulasuriya and Ariyawardana, 2016).

A study on the prevalence of OL, has reported that its onset takes place after the age of 30 years old; where OL may occur in men over 40 years of age, while in women over 50 years of age. Gender distribution however, varies in most studies. In addition, tobacco smoking has been the most commonly known

aetiology in the development of OL; with prevalence of smoking between 56 and 97 percent. Tobacco and alcohol consumption are known as coexistent factors with higher prevalence of OL in comparison to those who smoke tobacco only (Bokor-Bratic, 2003). The finding of this review is in accordance with those reported; with more than half of study patients with the age range of 40 to 50 years old, varying gender consisting of both male and female in majority of studies, as well as tobacco smoking and alcohol consumption were reported in a few studies.

To date, the predictive, diagnostic and prognostic marker(s) of OL is still unavailable. The risk of progression from OL to cancerous stage is determined by the presence of epithelial dysplasia on the tissue biopsy of the lesions (Parlatescu et al., 2014). Therefore, dysplastic features including cellular atypia and loss of normal stratification of OL specimens is an important characteristic to be observed thoroughly. This is usually done through the light and transmission electron microscopy. Dysplastic changes indeed, have been evaluated by three-fourth of the studies. It is, however, noteworthy, that the degree of dysplasia has also been shown to correlate with the chances of malignancy; from 4 to 11% for the mild or moderate dysplastic OL lesions, while 2 to 35% for severe dysplastic changes (Shirani et al., 2014). The stages of dysplasia including mild, moderate and severe, however, have been evaluated by less than one-third of the studies.

With regards to the genetic factors, the biomarkers involved in the formation and progression of OL that have been the focus of investigations include the cytokines, proliferation antigen, MHC and CD molecules, caspase, immune checkpoints, OSCC key genes, nuclear proteins, toll-like receptor, macrophage, polycomb complex, circular RNA and DEGs. The cytokines are able to transmit intercellular signal for cell transformation, the proliferation antigen and the OSCC key genes regulate cell cycle, the MHC and CD molecules aid in intercellular communication, the caspase controls inflammation and cell death, the immune checkpoints regulate immune system, the nuclear proteins play role as transcription factor, the toll-like receptor and macrophage play role in innate immune response and are able to initiate adaptive immune response, the polycomb complex regulates cell differentiation and proliferation, the circRNA regulates gene expression, while the DEGs differentiate the disease group from the healthy control. Of these biomarkers, the transcription factor p53 and the marker of cell proliferation Ki-67 have been evaluated by one-sixth of studies. This may be due to the well-accepted role of transcription factors in the modulation of signalling pathways leading to cell proliferation and p53 as the most known mutated gene in cancer, and proliferation antigen Ki-67 as a potential marker for determining the severity of oral epithelial dysplasia as it aid in the histological grading of OSCC.

In the present systematic review, various molecular biomarkers were detected in the tissues, which was further corroborated with its histopathological alteration. The results of the present systematic review, demonstrating an involvement of dysplasia in the epithelial layers of OL tissues towards its progression to OSCC, are in parallel of those in the previous systematic review which reported dysplasia as an associated risk factors involved in OL malignant transformation (Warnakulasuriya and Ariyawardana, 2016). With regards to the relationship between the biomarkers and their histopathological features of OL in terms of the risk for OSCC transformation, the present systematic review has found that overexpression of pro-inflammatory cytokines has a close relation with grades of OL differentiation towards OSCC. This may be due to the role of cytokines in the regulation of cell cycle, as well as immune surveillance and suppression. Previous studies have reported, that activation of pro-inflammatory cytokines is known to reduce the tumour suppressor activity of p53 (Levine, 2011) and mutant p53 prolongs the nuclear factor kappa B (NF-κB) activation and promotes chronic inflammation and inflammation-associated cancer (Uehara and Tanaka, 2018). Since pro-inflammatory cytokines can be modulated, thus the pro-inflammatory cytokines may therefore act as a potential biomarker for early detection, therapeutic target and prognostic marker of oral leukoplakia.

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CONCLUSION

The present systematic review shows that exposure to genetic alterations results in oral leukoplakia malignant transformation. Majority of the biomarkers are involved in cellular processes of dysplasia. In genetic involvement, cytokines play critical roles in OL transformation and progression in the early towards the advanced stages with close relation with dysplastic changes. Since cytokines are detectable in saliva, it could be a non-invasive biomarker for OL. Therefore, future studies on differential cytokines profiling of differentiated dysplasia, may reveal the novel stage-wise biomarkers for molecular pathological grading. The present review has provided important insights into oral leukoplakia etiopathogenesis, therapeutic targets and prognostic markers of therapy response.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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CONTRIBUTION OF AUTHORS

Noor Azliza Wani Abd. Aziz - Designed the study, collected data, wrote the manuscript. Corresponding author.

Jamil Ahsan Kazi - Designed the study, collected data, wrote the manuscript. First author.

Nur Hayani Batrisya Mohd Rosli - Collected data and performed the analysis.

Nur Sabrina Nazri - Collected data and performed the analysis.

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